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(71) Applicant: CREATIVE BIOMOLECULES, INC. [US/US]; 45 South Street, Hopkinton, MA 01748 (US).

(72) Inventors: RUEGER, David, C. ; 19 Downey Street, Hopkinton, MA 01748 (US). KUBERASAMPATH, Thangavel ; Six Spring Street, Medway, MA 02053 (US). OPPERMANN, Hermann ; 25 Summer Hill Road, Medway, MA 02053 (US). OZKAYNAK, Engin ; 44 Purdue Drive, Milford, MA 01757 (US). PANG, Roy, H., L. ; 15 Partridge Road, Etna, NH 03750 (US). COHEN, Charles, M. ; 98 Winthrop Street, Medway, MA 02053 (US).

(74) Agent: KELLEY, Robin, D.; Testa, Hurwitz & Thibeault, Exchange Place, 53 State Street, Boston, MA 02109 (US).

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(54) Title: MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR

(57) Abstract

Disclosed are therapeutic treatment methods, compositions and devices for maintaining neural pathways in a mammal, including enhancing survival of neurons at risk of dying, inducing cellular repair of damaged neurons and neural pathways, and stimulating neurons to maintain their differentiated phenotype. In one embodiment, the invention provides means for stimulating CAM expression in neurons. The invention also provides means for evaluating the status of nerve tissue, including means for detecting and monitoring neuropathies in a mammal. The methods, devices and compositions include a morphogen-stimulating agent provided to the mammal in a therapeutically effective concentration.

Morphogen-Induced Nerve Regeneration and Repair

5

BACKGROUND OF THE INVENTION

The present invention relates to methods for
enhancing the survival of neuronal cells in vivo and to
10 methods, compositions and devices for maintaining
neural pathways in vivo. More particularly, the
invention provides methods for enhancing survival of
neuronal cells at risk of dying, including methods for
redifferentiating transformed cells of neural origin
15 and methods for maintaining phenotypic expression of
differentiated neuronal cells. The invention also
provides means for repairing damaged neural pathways,
including methods for stimulating axonal growth over
extended distances, and methods for alleviating
20 immunologically-related nerve tissue damage. In a
particular embodiment of the invention, this invention
provides a method for stimulating cell adhesion
molecule expression in cells, and particularly nerve
cell adhesion molecule expression in neurons. Finally,
25 the invention provides means for evaluating nerve
tissue stasis and identifying neural dysfunction in a
mammal.

The mammalian nervous system comprises a peripheral
30 nervous system (PNS) and a central nervous system (CNS,
comprising the brain and spinal cord), and is composed
of two principal classes of cells: neurons and glial
cells. The glial cells fill the spaces between
neurons, nourishing them and modulating their function.
35 Certain glial cells, such as Schwann cells in the PNS

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and oligodendrocytes in the CNS, also provide a
protective myelin sheath that surrounds and protects
neuronal axons, which are the processes that extend
from the neuron cell body and through which the
5   electric impulses of the neuron are transported. In
the peripheral nervous system, the long axons of
multiple neurons are bundled together to form a nerve
or nerve fiber. These, in turn, may be combined into
fascicles, wherein the nerve fibers form bundles
10   embedded, together with the intraneural vascular
supply, in a loose collagenous matrix bounded by a
protective multilamellar sheath. In the central
nervous system, the neuron cell bodies are visually
distinguishable from their myelin-ensheathed processes,
15   and are referenced in the art as grey and white matter,
respectively.

During development, differentiating neurons from
the central and peripheral nervous systems send out
20   axons that must grow and make contact with specific
target cells. In some cases, growing axons must cover
enormous distances; some grow into the periphery,
whereas others stay confined within the central nervous
system. In mammals, this stage of neurogenesis is
25   complete during the embryonic phase of life and
neuronal cells do not multiply once they have fully
differentiated.

Accordingly, the neural pathways of a mammal are
30   particularly at risk if neurons are subjected to
mechanical or chemical trauma or to neuropathic
degeneration sufficient to put the neurons that define
the pathway at risk of dying. A host of neuropathies,
some of which affect only a subpopulation or a system
35   of neurons in the peripheral or central nervous systems

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have been identified to date. The neuropathies, which
may affect the neurons themselves or the associated
glial cells, may result from cellular metabolic
dysfunction, infection, exposure to toxic agents,
5   autoimmunity dysfunction, malnutrition or ischemia. In
some cases the cellular dysfunction is thought to
induce cell death directly. In other cases, the
neuropathy may induce sufficient tissue necrosis to
stimulate the body's immune/inflammatory system and the
10   mechanisms of the body's immune response to the initial
neural injury then destroys the neurons and the pathway
defined by these neurons.

Currently no satisfactory method exists to repair
15   the damage caused by these neuropathies, which include
multiple sclerosis, amyotrophic lateral sclerosis
(ALS), Huntington's chorea, Alzheimer's disease,
Parkinson's disease (parkinsonism), and metabolically
derived disorders, such as hepatic encephalopathy.
20   Current attempts to counteract the effects of severe
traumatic or neural degenerative lesions of the brain
and/or spinal cord have to date primarily involved
implantation of embryonic neurons in an effort to
replace functionally, or otherwise compensate for, lost
25   or deficient neurons. Currently, however, human fetal
cell transplantation research is severely restricted.
Administration of neurotrophic factors such as nerve
growth factor and insulin-like growth factor also have
been suggested to stimulate neuronal growth within the
30   CNS. (See, for example, Lundborg, (1987) Acta Orthop.
Scand. 58:145-169 and US Pat. No. 5,093,317.)
Administration of neurotrophic factors to the CNS
requires bypassing the blood-brain barrier. The
barrier may be overcome by direct infusion, or by
35   modifying the molecule to enhance its transport across

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the barrier, as by chemical modification or
conjugation, or by molecule truncation. Schwann cells
also have been grafted to a site of a CNS lesion in an
attempt to stimulate and maintain growth of damaged
5 neuronal processes (Paino et al. (1991) <u>Exp. Neurology</u>
<u>114</u>(2):254-257).

Where the damaged neural pathway results from CNS
axonal damage, autologous peripheral nerve grafts have
10 been used to bridge lesions in the central nervous
system and to allow axons to make it back to their
normal target area. In contrast to CNS neurons, neurons
of the peripheral nervous system can extend new
peripheral processes in response to axonal damage. This
15 regenerative property of peripheral nervous system
axons is thought to be sufficient to allow grafting of
these segments to CNS axons. Successful grafting
appears to be limited, however, by a number of factors,
including the length of the CNS axonal lesion to be
20 bypassed, and the distance of the graft sites from the
CNS neuronal cell bodies, with successful grafts
occurring near the cell body.

Within the peripheral nervous system, this cellular
25 regenerative property of neurons has limited ability to
repair function to a damaged neural pathway.
Specifically, the new axons extend randomly, and are
often misdirected, making contact with inappropriate
targets that can cause abnormal function. For example,
30 if a motor nerve is damaged, regrowing axons may
contact the wrong muscles, resulting in paralysis. In
addition, where severed nerve processes result in a gap
of longer than a few millimeters, e.g., greater than 10

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millimeters (mm), appropriate nerve regeneration does
not occur, either because the processes fail to grow
the necessary distance, or because of misdirected
axonal growth. Efforts to repair peripheral nerve
5   damage by surgical means has met with mixed results,
particularly where damage extends over a significant
distance. In some cases, the suturing steps used to
obtain proper alignment of severed nerve ends
stimulates the formulation of scar tissue which is
10   thought to inhibit axon regeneration. Even where scar
tissue formation has been reduced, as with the use of
nerve guidance channels or other tubular prostheses,
successful regeneration generally still is limited to
nerve damage of less than 10 millimeters in distance.
15   In addition, the reparative ability of peripheral
neurons is significantly inhibited where an injury or
neuropathy affects the cell body itself or results in
extensive degeneration of a distal axon.

20   Mammalian neural pathways also are at risk due to
damage caused by neoplastic lesions. Neoplasias of
both the neurons and glial cells have been identified.
Transformed cells of neural origin generally lose their
ability to behave as normal differentiated cells and
25   can destroy neural pathways by loss of function. In
addition, the proliferating tumors may induce lesions
by distorting normal nerve tissue structure, inhibiting
pathways by compressing nerves, inhibiting cerbrospinal
fluid or blood supply flow, and/or by stimulating the
30   body's immune response. Metastatic tumors, which are a
significant cause of neoplastic lesions in the brain
and spinal cord, also similarly may damage neural
pathways and induce neuronal cell death.

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    One type of morphoregulatory molecule associated
with neuronal cell growth, differentiation and
development is the cell adhesion molecule ("CAM"), most
notably the nerve cell adhesion molecule (N-CAM). CAMs
5 belong to the immunoglobulin super-family and mediate
cell-cell interactions in developing and adult tissues
through homophilic binding, i.e., CAM-CAM binding on
apposing cells. A number of different CAMs currently
have been identified. Of these, the most thoroughly
10 studied to date are N-CAM and L-CAM (liver cell
adhesion molecules), both of which have been identified
on all cells at early stages of development, as well as
in different adult tissues. In neural tissue
development, N-CAM expression is believed to be
15 important in tissue organization, neuronal migration,
nerve-muscle tissue adhesion, retinal formation,
synaptogenesis, and neural degeneration. Reduced N-CAM
expression also is thought to be associated with nerve
dysfunction. For example, expression of at least one
20 form of N-CAM, N-CAM-180, is reduced in a mouse
dysmyelinating mutant (Bhat (1988) _Brain_ _Res._ _452_:373-
377). Reduced levels of N-CAM also have been
associated with normal pressure hydrocephalus (Werdelin
(1989) _Acta_ _Neurol._ _Scand._ _79_:177-181), and with type
25 II schizophrenia (Lyons et al., (1988) _Biol._ _Psychiatry_
_23_:769-775.) In addition, antibodies to N-CAM have
been shown to disrupt functional recovery in injured
nerves (Remsen (1990) _Exp._ _Neurobiol._ _110_:268-273).

30   It is an object of this invention to provide
methods for enhancing survival of neurons at risk of
dying in a mammal. Another object is to provide
methods for maintaining neural pathways _in_ _vivo_ at risk
of injury, or following damage to nerve tissue due to
35 mechanical or chemical trauma, a neuropathy, or a

neoplastic lesion. Another object is to provide
compositions and devices for repairing gaps in a neural
pathway of the peripheral nervous system. Yet another
object is to provide a means for redifferentiating
5 transformed cells defining neural pathways,
particularly transformed cells of neural origin.
Another object is to provide a means for stimulating
CAM expression, particularly N-CAM expression in a
cell. Yet another object is to provide methods for
10 monitoring the status of nerve tissue by monitoring
fluctuations in protein levels present in nerve
tissue, serum and/or cerebrospinal fluid. These and
other objects and features of the invention will be
apparent from the description, drawings, and claims
15 which follow.

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Summary of the Invention

The present invention provides methods and
5   compositions for maintaining neural pathways in a
mammal in vivo, including methods for enhancing the
survival of neural cells.

In one aspect, the invention features compositions
10   and therapeutic treatment methods that comprise the
step of administering to a mammal a therapeutically
effective amount of a morphogenic protein
("morphogen"), as defined herein, upon injury to a
neural pathway, or in anticipation of such injury, for
15   a time and at a concentration sufficient to maintain
the neural pathway, including repairing damaged
pathways, or inhibiting additional damage thereto.

In another aspect, the invention features
20   compositions and therapeutic treatment methods for
maintaining neural pathways in a mammal in vivo which
include administering to the mammal, upon injury to a
neural pathway or in anticipation of such injury, a
compound that stimulates in vivo a therapeutically
25   effective concentration of an endogenous morphogen
within the body of the mammal sufficient to maintain
the neural pathway, including repairing damaged
pathways or inhibiting additional damage thereto.
These compounds are referred to herein as morphogen-
30   stimulating agents, and are understood to include
substances which, when administered to a mammal, act on
tissue(s) or organ(s) that normally are responsible

for, or capable of, producing a morphogen and/or
secreting a morphogen, and which cause the endogenous
level of the morphogen to be altered. The agent may
act, for example, by stimulating expression and/or
5   secretion of an endogenous morphogen.

In particular, the invention provides methods for
enhancing the survival of neurons at risk of dying,
including protecting neurons from the tissue
10   destructive effects associated with the body's immune/
inflammatory response to a nerve injury. The invention
also provides methods for stimulating neurons to
maintain their differentiated phenotype, including
inducing the redifferentiation of transformed cells of
15   neuronal origin to a morphology characteristic of
untransformed neurons. In one embodiment, the
invention provides means for stimulating production of
cell adhesion molecules in cells, particularly nerve
cell adhesion molecules (N-CAM) in neurons. The
20   invention also provides methods, compositions and
devices for stimulating cellular repair of damaged
neurons and neural pathways, including regenerating
damaged axons of the peripheral and central nervous
systems. In addition, the invention also provides
25   means for evaluating the status of nerve tissue, and
for detecting and monitoring neuropathies in a mammal
by monitoring fluctuations in the morphogen levels or
endogenous morphogen antibody levels present in a
mammal's serum or cerebrospinal fluid.
30

As used herein, a "neural pathway" describes a
nerve circuit for the passage of electric signals from
a source to a target cell site. The pathway includes
the neurons through which the electric impulse is·

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transported, including groups of interconnecting
neurons, the nerve fibers formed by bundled neuronal
axons, and the glial cells surrounding and associated
with the neurons.

5

In one aspect of the invention, the morphogens
described herein are useful in repairing damaged neural
pathways of the peripheral nervous system. In
particular, the morphogens are useful for repairing
10 damaged pathways, including transected or otherwise
damaged nerve fibers (nerves) requiring regeneration of
neuronal processes, particularly axons, over extended
distances to bridge a gap in the nerve itself, or
between the nerve and a post-synaptic cell.
15 Specifically, the morphogens described herein are
capable of stimulating complete axonal nerve
regeneration, including vascularization and reformation
of the protective myelin sheath. The morphogen
preferably is provided to the site of injury dispersed
20 in a biocompatible, bioresorbable carrier material
capable of maintaining the morphogen at the site and,
where necessary, means for directing axonal growth from
the proximal to the distal ends of a severed neuron or
nerve. For example, means for directing axonal growth
25 may be required where nerve regeneration is to be
induced over an extended distance, such as greater than
10 mm. Many carriers capable of providing these
functions are envisioned. For example, useful carriers
include substantially insoluble materials or viscous
30 solutions prepared as disclosed herein comprising
laminin, hyaluronic acid or collagen, or other suitable
synthetic, biocompatible polymeric materials such as
polylactic, polyglycolic or polybutyric acids and/or
copolymers thereof. The currently preferred carrier
35 comprises an extracellular matrix composition, such as

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one described herein derived, for example, from mouse
sarcoma cells. Also envisioned as especially useful
are brain tissue-derived extracellular matrices.

5   In a particularly preferred embodiment, the
morphogen is provided to the site as part of a device
wherein the morphogen is disposed in a nerve guidance
channel which spans the distance of the damaged
pathway. The channel acts both as a protective
10 covering and a physical means for guiding growth of a
neuronal process such as an axon. Useful channels
comprise a biocompatible membrane or casing, which may
be tubular in structure, having a dimension sufficient
to span the gap or break in the nerve to be repaired,
15 and having openings adapted to receive severed nerve
ends. The casing or membrane may be made of any
biocompatible, nonirritating material, such as silicone
or a biocompatible polymer such as polyethylene or
polyethylene vinyl acetate. The casing also may be
20 composed of biocompatible, bioresorbable polymers,
including, for example, collagen, hyaluronic acid,
polylactic, polybutyric and polyglycolic acids. In a
currently preferred embodiment, the outer surface of
the channel is substantially impermeable.
25
       The morphogen may be disposed in the channel in
association with a biocompatible carrier material, or
it may be adsorbed to or otherwise associated with the
inner surface of the casing, such as is described in
30 U.S. Pat. No. 5,011,486, provided that the morphogen is
accessible to the severed nerve ends. Additionally,
although the nerve guidance channels described herein
generally are tubular in shape, it should be evident to
those skilled in the art that various alternative
35 shapes may be employed. The lumen of the guidance

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channels may, for example, be oval or even square in
cross section. Moreover the guidance channels may be
constructed of two or more parts which may be clamped
together to secure the nerve stumps. Nerve endings may
5 be secured to the nerve guidance channels by means of
sutures, biocompatible adhesives such as fibrin glue,
or other means known in the medical art.

The morphogens described herein also are envisioned
10 to be useful in autologous peripheral nerve segment
implants to bypass damaged neural pathways in the
central nervous system, such as in the repair of
damaged or detached retinas, or other damage to the
optic nerve. Here the morphogen is provided to the
15 site of attachment to stimulate axonal growth at the
graft site, particularly where the damaged axonal
segment to be bypassed occurs far from the neuronal
cell body.

20   The morphogens described herein also are useful for
enhancing survival of neuronal cells at risk of dying,
thereby preventing, limiting or otherwise inhibiting
damage to neural pathways. Non-mitotic neurons are at
risk of dying as a result of a neuropathy or other
25 cellular dysfunction of a neuron or glial cell inducing
cell death, or following a chemical or mechanical
lesion to the cell or its surrounding tissue. The
chemical lesions may result from known toxic agents,
including lead, ethanol, ammonia, formaldehyde and many
30 other organic solvents, as well as the toxins in
cigarette smoke and opiates. Excitatory amino acids,
such as glutamate also may play a role in the
pathogenesis of neuronal cell death (see Freese et al.
(1990) Brain Res. 521:254-264). Neuronal cell death
35 also is thought to be a significant contributing factor

in a number of neurodegenerative diseases, including
Alzheimer's disease, Huntington's chorea, and
Parkinson's disease, amyotrophic lateral sclerosis and
multiple sclerosis. The etiology of these neuropathies
5   may be metabolic, as results in hepatic encephalopathy,
infectious, toxic, autoimmune, nutritional or ischemic.
In addition, ethanol and a number of other toxins also
have been identified as significant contributing
factors in neurodegenerative diseases. The morphogens
10   described herein may be provided to cells at risk of
dying to enhance their survival and thereby protect the
integrity of the neural pathway. The morphogens may be
provided directly to the site, or they may be provided
systemically. Alternatively, as described above, an
15   agent capable of stimulating endogenous morphogen
expression and/or secretion, preferably in cells
associated with the nerve tissue of interest, may be
administered to the mammal.

20   In another aspect of the invention, the method
disclosed is useful for redifferentiating transformed
cells, particularly transformed cells of neuronal or
glial origin, such that the morphogen-treated cells are
induced to display a morphology characteristic of
25   untransformed cells. Where the transformed cells are
cells of neuronal origin, morphogen treatment
preferably induces cell rounding and cell aggregation
(clumping), cell-cell adhesion, neurite outgrowth
formation and elongation, and N-CAM production. The
30   methods described herein are anticipated to
substantially inhibit or reduce neural cell tumor
formation and/or proliferation in nerve tissue. It is
anticipated that the methods of this invention will be
useful in substantially reducing the effects of various
35   carcinomas of nerve tissue origin such as

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retinoblastomas, neuroblastomas, and gliomas or
glioblastomas. In addition, the method also is
anticipated to aid in inhibiting neoplastic lesions
caused by metastatic tissue. Metastatic tumors are one
5   of the most common neoplasms of the CNS, as they can
reach the intracranial compartment through the
bloodstream. Metastatic tumors may damage neural
pathways for example, by distorting normal nerve tissue
structure, compressing nerves, blocking flow of
10   cerebrospinal fluid or the blood supply nourishing
brain tissue, and/or by stimulating the body's immune
response.

In another aspect of the invention, the morphogens
15   described herein are useful for providing
neuroprotective effects to alleviate neural pathway
damage associated with the body's immune/inflammatory
response to an initial injury to nerve tissue. Such a
response may follow trauma to nerve tissue, caused, for
20   example, by an autoimmune dysfunction, neoplastic
lesion, infection, chemical or mechanical trauma,
disease, by interruption of blood flow to the neurons
or glial cells, for example following ischemia or
hypoxia, or by other trauma to the nerve or surrounding
25   material. For example, the primary damage resulting
from hypoxia or ischemia-reperfusion following
occlusion of a neural blood supply, as in an embolic
stroke, is believed to be immunologically associated.
In addition, at least part of the damage associated
30   with a number of primary brain tumors also appears to
be immunologically related. Application of the
morphogen directly to the cells to be treated, or
providing the morphogen to the mammal systemically, for
example, intravenously or indirectly by oral
35   administration, may be used to alleviate and/or inhibit

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the immunologically related response to a neural
injury. Alternatively, administration of an agent
capable of stimulating morphogen expression and/or
secretion in vivo, preferably at the site of injury,
5   also may be used. Where the injury is to be induced,
as during surgery or other aggressive clinical
treatment, the morphogen or agent may be provided prior
to induction of the injury to provide a neuroprotective
effect to the nerve tissue at risk.
10

In still another aspect, the invention described
herein provides methods for supporting the growth and
maintenance of differentiated neurons, including
inducing neurons to continue expressing their
15   phenotype. It is anticipated that this activity will
be particularly useful in the treatment of nerve tissue
disorders where loss of function is caused by reduced
or lost cellular metabolic function and cells become
senesent or quiescent, such as is thought to occur in
20   aging cells and to be manifested in Alzheimer's
disease. Application of the morphogen directly to
cells to be treated, or providing it systemically by
parenteral or oral administration stimulates these
cells to continue expressing their phenotype,
25   significantly inhibiting and/or reversing the effects
of the cellular metabolic dysfunction, thereby
maintaining the neural pathway at risk. Alternatively,
administration of an agent capable of stimulating
endogenous morphogen expression and/or secretion in
30   vivo may be used.

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In still another aspect, the invention provides
methods for stimulating CAM expression levels in a
cell, particularly N-CAM expression in neurons. CAMs
are molecules defined as carrying out cell-cell
5   interactions necessary for tissue formation. CAMs are
believed to play a fundamental regulatory role in
tissue development, including tissue boundary
formation, embryonic induction and migration, and
tissue stabilization and regeneration. Altered CAM
10   levels have been implicated in a number of tissue
disorders, including congenital defects, neoplasias,
and degenerative diseases.

In particular, N-CAM expression is associated with
15   normal neuronal cell development and differentiation,
including retinal formation, synaptogenesis, and nerve-
muscle tissue adhesion. Inhibition of one or more of
the N-CAM isoforms is known to prevent proper tissue
development. Altered N-CAM expression levels also are
20   associated with neoplasias, including neuroblastomas
(see infra), as well as with a number of neuropathies,
including normal pressure hydrocephalous and type II
schizophrenia. Application of the morphogen directly
to the cells to be treated, or providing the morphogen
25   to the mammal systemically, for example, parenterally,
or indirectly by oral administration, may be used to
induce cellular expression of one or more CAMs,
particularly N-CAMs. Alternatively, administration of
an agent capable of stimulating morphogen expression
30   and/or secretion in vivo, preferably at the site of
injury, also may be used to induce CAM production.

CAMs also have been postulated as part of a
morphoregulatory pathway whose activity is induced by a
35   to date unidentified molecule (See, for example,

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Edelman, G.M. (1986) <u>Ann. Rev. Cell Biol.</u> 2:81-116).
Without being limited to any given theory, the
morphogens described herein may act as the inducer of
this pathway.

5

Finally, modulations of endogenous morphogen levels
may be monitored as part of a method of detecting nerve
tissue dysfunction. Specifically, modulations in
endogenous morphogen levels are anticipated to reflect
10 changes in nerve tissue status. Morphogen expression
may be monitored directly in biopsied cell samples, in
cerebrospinal fluid, or serum. Alternatively,
morphogen levels may be assessed by detecting changes
in the levels of endogenous antibodies to the
15 morphogen. For example, one may obtain serum samples
from a mammal, and then detect the concentration of
morphogen or antibody present in the fluid by standard
protein detection means known to those skilled in the
art. As an example, binding protein capable of
20 interacting specifically with the morphogen of interest
such as an anti-morphogen antibody may be used to
detect a morphogen in a standard immunoassay. The
morphogen levels detected then may be compared to a
previously determined standard or reference level, with
25 changes in the detected levels being indicative of the
status of the tissue.

In one preferred embodiment of the invention, the
morphogen or morphogen-stimulating agent is
30 administered systemically to the individual, e.g.,
orally or parenterally. In another embodiment of the
invention, the morphogen may be provided directly to
the nerve tissue, e.g., by injection to the cerebral
spinal fluid or to a nerve tissue locus.

35

In any treatment method of the invention,
"administration of morphogen" refers to the
administration of the morphogen, either alone or in
combination with other molecules. For example, the
5   mature form of the morphogen may be provided in
association with its precursor "pro" domain, which is
known to enhance the solubility of the protein. Other
useful molecules known to enhance protein solubility
include casein and other milk components, as well as
10 various serum proteins. Additional useful molecules
which may be associated with the morphogen or
morphogen-stimulating agent include tissue targeting
molecules capable of directing the morphogen or
morphogen-stimulating agent to nerve tissue. Tissue
15 targeting molecules envisioned to be useful in the
treatment protocols of this invention include
antibodies, antibody fragments or other binding
proteins which interact specifically with surface
molecules on nerve tissue cells.
20

Still another useful tissue targeting molecule is
part or all of the morphogen precursor "pro" domain,
particularly that of OP-1 or GDF-1. These proteins are
found naturally associated with nerve tissue but also
25 may be synthesized in other tissues and targeted to
nerve tissue after secretion from the synthesizing
tissue. For example, while the protein has been shown
to be active in bone tissue, the primary source of OP-1
synthesis appears to be the tissue of the urogenic
30 system (e.g., renal and bladder tissue), with secondary
expression levels occurring in the brain, heart and
lungs (see below.) Moreover, the protein has been
identified in serum, saliva and various milk forms. In
addition, the secreted form of the protein comprises
35 the mature dimer in association with the pro domain of

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the intact morphogen sequence. Accordingly, the associated morphogen pro domains may act to target specific morphogens to different tissues in vivo.

5   Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of
10   bone remodeling sites.

Finally, the morphogens or morphogen-stimulating agents provided herein also may be administered in combination with other molecules known to be beneficial
15   in maintaining neural pathways, including, for example, nerve growth factors and anti-inflammatory agents.

Where the morphogen is intended for use as a therapeutic for disorders of the CNS, an additional
20   problem must be addressed: overcoming the so-called "blood-brain barrier", the brain capillary wall structure that effectively screens out all but selected categories of molecules present in the blood, preventing their passage into the brain. The
25   blood-brain barrier may be bypassed effectively by direct infusion of the morphogen or morphogen-stimulating agent into the brain. Alternatively, the morphogen or morphogen-stimulating agent may be modified to enhance its transport across the
30   blood-brain barrier. For example, truncated forms of the morphogen or a morphogen-stimulating agent may be most successful. Alternatively, the morphogen or

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morphogen-stimulating agent may be modified to render
it more lipophilic, or it may be conjugated to another
molecule which is naturally transported across the
barrier, using standard means known to those skilled in
5 the art, as, for example, described in Pardridge,
Endocrine Reviews 7:314-330 (1986) and U.S. Pat.
No. 4,801,575.

Accordingly, as used herein, a functional "analog"
10 of a morphogen refers to a protein having morphogenic
biological activity but possessing additional
structural differences compared to a morphogen as
defined herein, e.g., having additional chemical
moieties not normally a part of a morphogen. Such
15 moieties (introduced, for example, by acylation,
alkylation, cationization, or glycosylation reactions,
or other means for conjugating the moiety to the
morphogen) may improve the molecule's solubility,
absorption, biological half-life, or transport, e.g.,
20 across the blood-brain barrier.

Among the morphogens useful in this invention
are proteins originally identified as osteogenic
proteins, such as the OP-1, OP-2 and CBMP2 proteins, as
25 well as amino acid sequence-related proteins such as
DPP (from Drosophila), Vgl (from Xenopus), Vgr-1 (from
mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1
(from mouse, see Lee (1991) PNAS 88:4250-4254), all of
which are presented in Table II and Seq. ID Nos.5-14),
30 and the recently identified 60A protein (from
Drosophila, Seq. ID No. 24, see Wharton et al. (1991)
PNAS 88:9214-9218.) The members of this family, which
include members of the TGF-β super-family of proteins,
share substantial amino acid sequence homology in their
35 C-terminal regions.   The proteins are translated as a

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precursor, having an N-terminal signal peptide
sequence, typically less tahn about 30 residues,
followed by a "pro" domain that is cleaved to yield the
mature sequence. The signal peptide is cleaved rapidly
5   upon translation, at a cleavage site that can be
predicted in a given sequence using the method of Von
Heijne ((1986) <u>Nucleic Acids Research</u> <u>14</u>:4683-4691.)
Table I, below, describes the various morphogens
identified to date, including their nomenclature as
10   used herein, their Seq. ID references, and publication
sources for the amino acid sequences for the full
length proteins not included in the Seq. Listing. The
disclosure of these publications is incorporated herein
by reference.

15   TABLE I

"OP-1"   Refers generically to the group of
morphogenically active proteins expressed
from part or all of a DNA sequence
20   encoding OP-1 protein, including allelic
and species variants thereof, e.g., human
OP-1 ("hOP-1", Seq. ID No. 5, mature
protein amino acid sequence), or mouse
OP-1 ("mOP-1", Seq. ID No. 6, mature
25   protein amino acid sequence.) The
conserved seven cysteine skeleton is
defined by residues 38 to 139 of Seq. ID
Nos. 5 and 6. The cDNA sequences and the
amino acids encoding the full length
30   proteins are provided in Seq. Id Nos. 16
and 17 (hOP1) and Seq. ID Nos. 18 and 19
(mOP1.) The mature proteins are defined
by residues 293-431 (hOP1) and 292-430
(mOP1). The "pro" regions of the
35   proteins, cleaved to yield the mature,

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morphogenically active proteins are
defined essentially by residues 30-292
(hOP1) and residues 30-291 (mOP1).

5   "OP-2"   refers generically to the group of active
proteins expressed from part or all of a
DNA sequence encoding OP-2 protein,
including allelic and species variants
thereof, e.g., human OP-2 ("hOP-2", Seq.
10   ID No. 7, mature protein amino acid
sequence) or mouse OP-2 ("mOP-2", Seq. ID
No. 8, mature protein amino acid
sequence). The conserved seven cysteine
skeleton is defined by residues 38 to 139
15   of Seq. ID Nos. 7 and 8. The cDNA
sequences and the amino acids encoding the
full length proteins are provided in Seq.
ID Nos. 20 and 21 (hOP2) and Seq. ID Nos.
22 and 23 (mOP2.) The mature proteins are
20   defined essentially by residues 264-402
(hOP2) and 261-399 (mOP2). The "pro"
regions of the proteins, cleaved to yield
the mature, morphogenically active
proteins likely are defined essentially by
25   residues 18-263 (hOP2) and residues 18-260
(mOP2). (Another cleavage site also
occurs 21 residues upstream for both OP-2
proteins.)

30   "CBMP2"   refers generically to the morphogenically
active proteins expressed from a DNA
sequence encoding the CBMP2 proteins,
including allelic and species variants
thereof, e.g., human CBMP2A ("CBMP2A(fx)",
35   Seq ID No. 9) or human CBMP2B DNA

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("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear

5   in Wozney, et al. (1988) <u>Science</u> <u>242</u>:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B)

10   likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

"DPP(fx)"   refers to protein sequences encoded by the
15   Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) <u>Nature</u> <u>325</u>: 81-84. The pro

20   domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

25 "Vgl(fx)"   refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in

30   Weeks (1987) <u>Cell</u> <u>51</u>: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

35

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"Vgr-1(fx)"   refers to protein sequences encoded by the
                 murine Vgr-1 gene and defining the
                 conserved seven cysteine skeleton (Seq. ID
                 No. 13). The amino acid sequence for the
5   full length protein appears in Lyons, et
                 al, (1989) PNAS 86: 4554-4558. The
                 prodomain likely extends from the signal
                 peptide cleavage site to residue 299; the
                 mature protein likely is defined by
10   residues 300-438.

"GDF-1(fx)"   refers to protein sequences encoded by the
                 human GDF-1 gene and defining the
                 conserved seven cysteine skeleton (Seq. ID
15   No. 14). The cDNA and encoded amino
                 sequence for the full length protein is
                 provided in Seq. ID. No. 32. The
                 prodomain likely extends from the signal
                 peptide clavage site to residue 214; the
20   mature protein likely is defined by
                 residues 215-372.

"60A"   refers generically to the morphogenically
                 active proteins expressed from part or all
25   of a DNA sequence (from the Drosophila 60A
                 gene) encoding the 60A proteins (see Seq.
                 ID No. 24 wherein the cDNA and encoded
                 amino acid sequence for the full length
                 protein is provided). "60A(fx)" refers to
30   the protein sequences defining the
                 conserved seven cysteine skeleton
                 (residues 354 to 455 of Seq. ID No. 24.)

- 25 -

The prodomain likely extends from the
signal peptide cleavage site to residue
324; the mature protein likely is defined
by residues 325-455.

"BMP3(fx)"   refers to protein sequences encoded by the
human BMP3 gene and defining the conserved
seven cysteine skeleton (Seq. ID No. 26).
The amino acid sequence for the full
length protein appears in Wozney et al.
(1988) Science 242: 1528-1534. The pro
domain likely extends from the signal
peptide cleavage site to residue 290; the
mature protein likely is defined by
residues 291-472.

"BMP5(fx)"   refers to protein sequences encoded by the
human BMP5 gene and defining the conserved
seven cysteine skeleton (Seq. ID No. 27).
The amino acid sequence for the full
length protein appears in Celeste, et al.
(1991) PNAS 87: 9843-9847. The pro domain
likely extends from the signal peptide
cleavage site to residue 316; the mature
protein likely is defined by residues
317-454.

"BMP6(fx)"   refers to protein sequences encoded by the
human BMP6 gene and defining the conserved
seven cysteine skeleton (Seq. ID No. 28).
The amino acid sequence for the full
length protein appear sin Celeste, et al.

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(1990) <u>PNAS</u> 87: 9843-5847. The pro domain
likely includes extends from the signal
peptide cleavage site to residue 374; the
mature sequence likely includes
5   residues 375-513.

The OP-2 proteins have an additional cysteine
residue in this region (e.g., see residue 41 of Seq. ID
10   Nos. 7 and 8), in addition to the conserved cysteine
skeleton in common with the other proteins in this
family. The GDF-1 protein has a four amino acid insert
within the conserved skeleton (residues 44-47 of Seq.
ID No. 14) but this insert likely does not interfere
15   with the relationship of the cysteines in the folded
structure. In addition, the CBMP2 proteins are missing
one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are
20   active as oxidized homodimers and when oxidized in
combination with other morphogens of this invention.
Thus, as defined herein, a morphogen is a dimeric
protein comprising a pair of polypeptide chains,
wherein each polypeptide chain comprises at least the
25   C-terminal six cysteine skeleton defined by residues
43-139 of Seq. ID No. 5, including functionally
equivalent arrangements of these cysteines (e.g., amino
acid insertions or deletions which alter the linear
arrangement of the cysteines in the sequence but not
30   their relationship in the folded structure), such that,
when the polypeptide chains are folded, the dimeric
protein species comprising the pair of polypeptide
chains has the appropriate three-dimensional structure,
including the appropriate intra- or inter-chain
35   disulfide bonds such that the protein is capable of

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acting as a morphogen as defined herein. Specifically,
the morphogens generally are capable of all of the
following biological functions in a morphogenically
permissive environment: stimulating proliferation of
5   progenitor cells; stimulating the differentiation of
progenitor cells; stimulating the proliferation of
differentiated cells; and supporting the growth and
maintenance of differentiated cells. In addition, it
is also anticipated that these morphogens are capable
10 of inducing redifferentiation of committed cells under
appropriate environmental conditions.

In one preferred aspect, the morphogens of
this invention comprise one of two species of generic
15 amino acid sequences: Generic Sequence 1 (Seq. ID
No. 1) or Generic Sequence 2 (Seq. ID No. 2); where
each Xaa indicates one of the 20 naturally-occurring
L-isomer, α-amino acids or a derivative thereof.
Generic Sequence 1 comprises the conserved six cysteine
20 skeleton and Generic Sequence 2 comprises the conserved
six cysteine skeleton plus the additional cysteine
identified in OP-2 (see residue 36, Seq. ID No. 2). In
another preferred aspect, these sequences further
comprise the following additional sequence at their N-
25 terminus:

Cys Xaa Xaa Xaa Xaa   (Seq. ID No. 15)
1   5

30   Preferred amino acid sequences within the
foregoing generic sequences include: Generic Sequence
3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4),
Generic Sequence 5 (Seq. ID No. 30) and Generic
Sequence 6 (Seq. ID No. 31), listed below. These

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Generic Sequences accommodate the homologies shared
among the various preferred members of this morphogen
family identified in Table II, as well as the amino
acid sequence variation among them. Specifically,
5 Generic Sequences 3 and 4 are composite amino acid
sequences of the following proteins presented in
Table II and identified in Seq. ID Nos. 5-14: human
OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1
(mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2
10 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9),
CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID
No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1
(from mouse, Seq. ID No. 13), and GDF-1 (from mouse,
Seq. ID No. 14.) The generic sequences include both
15 the amino acid identity shared by the sequences in
Table II, as well as alternative residues for the
variable positions within the sequence. Note that
these generic sequences allow for an additional
cysteine at position 41 or 46 in Generic Sequences 3 or
20 4, respectively, providing an appropriate cysteine
skeleton where inter- or intramolecular disulfide bonds
can form, and contain certain critical amino acids
which influence the tertiary structure of the proteins.

25   Generic Sequence 3
             Leu Tyr Val Xaa Phe

             1   5

         Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

                         10

30   Xaa Ala Pro Xaa Gly Xaa Xaa Ala

             15   20

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```
      Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
              25                  30

      Xaa Pro Xaa Xaa Xaa Xaa Xaa
                      35

5     Xaa Xaa Xaa Asn His Ala Xaa Xaa
              40                  45

      Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                      50

      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
10        55                  60

      Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                  65

      Xaa Xaa Xaa Leu Xaa Xaa Xaa
      70                  75

15    Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
                  80

      Xaa Xaa Xaa Xaa Met Xaa Val Xaa
      85                  90

      Xaa Cys Gly Cys Xaa
20                95
```

wherein each Xaa is independently selected from a group
of one or more specified amino acids defined as
follows: "Res." means "residue" and Xaa at res.4 =
(Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or
25 Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu
or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn);
Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile
or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =

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(Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr
or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu
or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at
res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =
5   (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro
or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala
or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala);
Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at
10   res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn
or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at
res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or
Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 =
(Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at
15   res.49 = (Val or Met); Xaa at res.50 = (His or Asn);
Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa
at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53
= (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser);
Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56
20   = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at
res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or
Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 =
(Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at
res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg
25   or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at
res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro
or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at
res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met);
Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr
30   or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 =
(Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn
or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at
res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or
Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 =
35   (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His);

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Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 =
(Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala);
Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at
res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or
5 Arg);

Generic Sequence 4

```
        Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
10       1               5                   10
        Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                        15
        Xaa Ala Pro Xaa Gly Xaa Xaa Ala
        20                  25
15      Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                    30              35
        Xaa Pro Xaa Xaa Xaa Xaa Xaa
                        40
        Xaa Xaa Xaa Asn His Ala Xaa Xaa
20                  45              50
        Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
                        55
        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
            60                  65
25      Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                        70
        Xaa Xaa Xaa Leu Xaa Xaa Xaa
        75                  80
        Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
30                      85
        Xaa Xaa Xaa Xaa Met Xaa Val Xaa
            90                  95
        Xaa Cys Gly Cys Xaa
                    100
```

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wherein each Xaa is independently selected from a group
of one or more specified amino acids as defined by the
following: "Res." means "residue" and Xaa at res.2 =
(Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4

5   = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg
or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at
res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp
or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 =
(Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg,

10 or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 =
(Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro
or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 =
(Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 =
(Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp

15 or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at
res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu
or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 =
(Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu,
Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or

20 Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at
res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu
or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 =
(Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at
res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or

25 Met); Xaa at res.55 = (His or Asn); Xaa at res.56 =
(Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile,
Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala
or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 =
(Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val,

30 Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala
or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 =
(Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at
res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala);
Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 =

35 (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp);

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Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 =
(Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at
res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe);
Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp

5   or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at
res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser,
Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys);
Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or
Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at

10 res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or
Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95
= (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val,
Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa
at res.102 = (His or Arg).

15

    Similarly, Generic Sequence 5 (Seq. ID No. 30) and
Generic Sequence 6 (Seq. ID No. 31) accommodate the
homologies shared among all the morphogen protein
family members identified in Table II. Specifically,

20 Generic Sequences 5 and 6 are composite amino acid
sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-
17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19),
human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22),
CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP

25 (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus,
Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13),
and GDF-1 (from mouse, Seq. ID No. 14), human BMP3
(Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human
BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq.

30 ID Nos. 24-25). The generic sequences include both the
amino acid identity shared by these sequences in the
C-terminal domain, defined by the six and seven
cysteine skeltons (Generic Sequences 5 and 6,
respectively), as well as alternative residues for the

35 variable positions within the sequence. As for Generic

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Sequences 3 and 4, Generic Sequences 5 and 6 allow for
an additional cysteine at position 41 (Generic Sequence
5) or position 46 (Generic Sequence 6), providing an
appropriate cysteine skeleton where inter- or
5   intramolecular disulfide bonds can form, and containing
certain critical amino acids which influence the
tertiary structure of the proteins.

<u>Generic Sequence 5</u>

10

       Leu Xaa Xaa Xaa Phe

       1   5

    Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

               10

15   Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala

     15   20

    Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

        25   30

    Xaa Pro Xaa Xaa Xaa Xaa Xaa

20   35

    Xaa Xaa Xaa Asn His Ala Xaa Xaa

        40   45

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

              50

25   Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

     55   60

    Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

        65

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          Xaa Xaa Xaa Leu Xaa Xaa Xaa

          70   75

          Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

                      80

   5   Xaa Xaa Xaa Xaa Met Xaa Val Xaa

          85   90

          Xaa Cys Xaa Cys Xaa

                   95

        wherein each Xaa is independently selected from a group
   10   of one or more specified amino acids defined as
        follows: "Res." means "residue" and Xaa at res.2 =
        (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4
        = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys
        or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8
   15   = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp,
        His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or
        Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 =
        (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18
        = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 =
   20   (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at
        res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly);
        Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 =
        (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu,
        Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro,
   25   Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
        res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp,
        Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu,
        Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or
        Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at
   30   res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser,
        Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at
        res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu

or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 =
(Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at
res.49 = (Val or Met); Xaa at res.50 = (His, Asn or
Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val);
5   Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa
at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at
res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp,
Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val,
Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 =
10   (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at
res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or
Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 =
(Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or
Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68
15   = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or
Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at
res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met
or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 =
(Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or
20   Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at
res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 =
(Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr
or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at
res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln,
25   His or Val); Xaa at res.86 = (Tyr or His); Xaa at
res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn,
Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile);
Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at
res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly
30   or Ala) and Xaa at res.97 = (His or Arg).

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Generic Sequence 6

```
        Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
        1               5                   10
5       Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                        15
        Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
        20              25
        Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
10          30                  35
        Xaa Pro Xaa Xaa Xaa Xaa Xaa
                        40
        Xaa Xaa Xaa Asn His Ala Xaa Xaa
                45                  50
15      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                        55
        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
            60                  65
        Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
20                  70
        Xaa Xaa Xaa Leu Xaa Xaa Xaa
        75                  80
        Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
                        85
25      Xaa Xaa Xaa Xaa Met Xaa Val Xaa
        90                  95
        Xaa Cys Xaa Cys Xaa
                100
```

30   wherein each Xaa is independently selected from a group
      of one or more specified amino acids as defined by the
      following: "Res." means "residue" and Xaa at res.2 =
      (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or
      Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 =
35   (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at

res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa
at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg,
Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or
Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16
5 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 =
(Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val);
Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or
Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg);
Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or
10 Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln,
Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at
res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at
res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 =
(Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu
15 or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at
res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 =
(Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,
Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly
or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at
20 res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,
Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at
res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53
= (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at
25 res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,
Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =
30 (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro
or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =
35 (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or

Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at
res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr,
Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at
res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or

5   Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at
res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu,
Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr 'or
Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa
at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile,

10   Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at
res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 =
(Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro);
Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 =
(Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val,

15   Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser);
Xaa at res.100 = (Gly or Ala); and Xaa at res.102 =
(His or Arg).

          Particularly useful sequences for use as
20   morphogens in this invention include the C-terminal
domains, e.g., the C-terminal 96-102 amino acid
residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A,
CBMP-2B, GDF-1 (see Table II, below, and Seq. ID
Nos. 5-14), as well as proteins comprising the

25   C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see
Seq. ID Nos. 24-28), all of which include at least the
conserved six or seven cysteine skeleton. In addition,
biosynthetic constructs designed from the generic
sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S.

30   Pat. No. 5,011,691, also are useful. Other sequences
include the inhibins/activin proteins (see, for
example, U.S. Pat. Nos. 4,968,590 and 5,011,691).
Accordingly, other useful proteins are those exhibiting
morphogenic activity and having amino acid sequences

35   sharing at least 70% amino acid sequence homology or

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"similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic variants, species variants and other sequence variants (e.g., "muteins" or "mutant
5   proteins"), whether naturally occurring or biosynthetically produced, as well as novel members of this morphogenic family of proteins.

     As used herein, "amino acid sequence homology" is
10 understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are conserved amino acids as defined by Dayoff et al., <u>Atlas of Protein Sequence and Structure</u>; vol.5, Suppl.3, pp.345-362
15 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of
20 the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned
25 sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the
30 corresponding amino acid in the reference sequence.

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As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et
5 al. (1970) J.Mol. Biol. 48:443-453 and identities calculated by the Align program (DNAstar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.
10

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence
15 defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in another
20 preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various
25 identified species of OP1 and OP2 (Seq. ID No. 29).

In still another preferred aspect of the invention, useful morphogens include active proteins comprising polypeptide chains encoded by nucleic acids which
30 hybridize to DNA or RNA sequences encoding the C-terminal sequence defining the consumed cysteine domain, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. Id. Nos. 16 and 20, respectively, of OP1 or OP2 under stringent hybridization conditions.
35 As used herein, stringent hybridization conditions are

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defined as hybridization in 40% formamide, 5 X SSPE,
5 X Denhardt's Solution, and 0.1% SDS at 37°C
overnight, and wshing in 0.1 X SSPE, 0.1% SDS at 50°C.

5   The morphogens useful in the methods, composition
and devices of this invention include proteins
comprising any of the polypeptide chains described
above, whether isolated from naturally-occurring
sources, or produced by recombinant DNA or other
10   synthetic techniques, and includes allelic and species
variants of these proteins, naturally-occurring or
biosynthetic mutants thereof, as well as various
truncated and fusion constructs. Deletion or addition
mutants also are envisioned to be active, including
15   those which may alter the conserved C-terminal cysteine
skeleton, provided that the alteration does not
functionally disrupt the relationship of these
cysteines in the folded structure. Accordingly, such
active forms are considered the equivalent of the
20   specifically described constructs disclosed herein.
The proteins may include forms having varying
glycosylation patterns, varying N-termini, a family of
related proteins having regions of amino acid sequence
homology, and active truncated or mutated forms of
25   native or biosynthetic proteins, produced by expression
of recombinant DNA in host cells.

The morphogenic proteins can be expressed from
intact or truncated cDNA or from synthetic DNAs in
30   procaryotic or eucaryotic host cells, and purified,
cleaved, refolded, and dimerized to form
morphogenically active compositions. Currently
preferred host cells include E. coli or mammalian
cells, such as CHO, COS or BSC cells. A detailed
35   description of the morphogens useful in the methods,

compositions and devices of this invention is disclosed
in copending US patent application Serial Nos. 752,764,
filed August 30, 1991, and 667,274, filed March 11,
1991, the disclosure of which are incorporated herein
5   by reference.

Thus, in view of this disclosure, skilled genetic
engineers can isolate genes from cDNA or genomic
libraries of various different species which encode
10 appropriate amino acid sequences, or construct DNAs
from oligonucleotides, and then can express them in
various types of host cells, including both procaryotes
and eucaryotes, to produce large quantities of active
proteins capable of maintaining neural pathways in a
15 mammal, including enhancing the survival of neurons at
risk of dying and stimulating nerve regeneration and
repair in a variety of mammals, including humans.

The foregoing and other objects, features and
20 advantages of the present invention will be made more
apparent from the following detailed description of the
invention.

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Brief Description of the Drawings:

The foregoing and other objects and features of
this invention, as well as the invention itself, may be
5 more fully understood from the following description,
when read together with the accompanying drawings, in
which:

Fig. 1(A and B) are photomicrographs illustrating
10 the ability of morphogen (OP-1) to induce transformed
neuroblastoma x glioma cells (1A) to redifferentiate to
a morphology characteristic of untransformed neurons
(1B);

15   Fig. 2A is a dose response curve for the induction
of the 180 kDa and 140 kDa N-CAM isoforms in morphogen-
treated NG108-15 cells;

Fig. 2B is a photomicrograph of a Western blot of
20 whole cell extracts from morphogen-treated NG108-15
cells with an N-CAM-specific antibody; and

Fig. 3 is a plot of the mean number of cell
aggregates counted in 20 randomly selected fields as a
25 function of morphogen concentration.

Fig. 4 is a photomicrograph of an immunoblot
demonstrating the presence of OP-1 in human serum.

30

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Detailed Description of the Invention

It now has been discovered that the proteins
described herein are effective agents for enhancing the
5 survival of neurons, particularly neurons at risk of
dying, and for maintaining neural pathways in a mammal.
As described herein, these proteins ("morphogens") are
capable of enhancing survival of non-mitotic neurons,
stimulating neuronal CAM expression, maintaining the
10 phenotypic expression of differentiated neurons,
inducing the redifferentiation of transformed cells of
neural origin, and stimulating axonal growth over
breaks in neural processes, particularly large gaps in
distal axons. The proteins also are capable of
15 providing a neuroprotective effect to alleviate the
tissue destructive effects associated with
immunologically-related nerve tissue damage. Finally,
the proteins may be used as part of a method for
monitoring the viability of nerve tissue in a mammal.
20

Provided below are detailed descriptions of
suitable morphogens useful in the methods, compositions
and devices of this invention, as well as methods for
their administration and application, and numerous,
25 nonlimiting examples which 1) illustrate the
suitability of the morphogens and morphogen-stimulating
agents described herein as therapeutic agents for
maintaining nerual pathways in a mammal and enhancing
survival of neuronal cells at risk of dying; and
30 2) provide assays with which to test candidate
morphogens and morphogen-stimulating agents for their
efficacy.

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I. Useful Morphogens

As defined herein a protein is morphogenic if it is
capable of inducing the developmental cascade of
5 cellular and molecular events that culminate in the
formation of new, organ-specific tissue and comprises
at least the conserved C-terminal six cysteine skeleton
or its functional equivalent (see supra).
Specifically, the morphogens generally are capable of
10 all of the following biological functions in a
morphogenically permissive environment: stimulating
proliferation of progenitor cells; stimulating the
differentiation of progenitor cells; stimulating the
proliferation of differentiated cells; and supporting
15 the growth and maintenance of differentiated cells.
Details of how the morphogens useful in the method of
this invention first were identified, as well as a
description on how to make, use and test them for
morphogenic activity are disclosed in international
20 application US92/01968 (WO92/15323), the disclosure of
which is hereby incorporated by reference. As
disclosed therein, the morphogens may be purified from
naturally-sourced material or recombinantly produced
from procaryotic or eucaryotic host cells, using the
25 genetic sequences disclosed therein. Alternatively,
novel morphogenic sequences may be identified following
the procedures disclosed therein.

Particularly useful proteins include those which
30 comprise the naturally derived sequences disclosed in
Table II. Other useful sequences include biosynthetic
constructs such as those disclosed in U.S. Pat.

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5,011,691, the disclosure of which is incorporated
herein by reference (e.g., COP-1, COP-3, COP-4, COP-5,
COP-7, and COP-16).

5   Accordingly, the morphogens useful in the methods
and compositions of this invention also may be
described by morphogenically active proteins having
amino acid sequences sharing 70% or, preferably, 80%
homology (similarity) with any of the sequences
10 described above, where "homology" is as defined herein
above.

The morphogens useful in the method of this
invention also can be described by any of the 6 generic
15 sequences described herein (Generic Sequences 1, 2, 3,
4, 5 and 6). Generic sequences 1 and 2 also may
include, at their N-terminus, the sequence

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
20   1   5

Table II, set forth below, compares the amino acid
sequences of the active regions of native proteins that
have been identified as morphogens, including human
25 OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1
(mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2
(Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9),
CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP
(from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus,
30 Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13),
GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A
protein (from Drosophila, Seq. ID Nos. 24 and 25), BMP5
(Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The
sequences are aligned essentially following the method
35 of Needleman et al. (1970) J. Mol. Biol., 48:443-453,

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calculated using the Align Program (DNAstar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	
mOP-1	...	...	...	...	...	...	...	...	
hOP-2	...	Arg	Arg	...	...	...	...	...	
mOP-2	...	Arg	Arg	...	...	...	...	...	
DPP	...	Arg	Arg	...	Ser	...	...	...	
Vgl	...	...	Lys	Arg	His	...	...	...	
Vgr-1	...	...	...	...	Gly	...	...	...	
CBMP-2A	...	...	Arg	...	Pro	...	...	...	
CBMP-2B	...	Arg	Arg	...	Ser	...	...	...	
BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...	
GDF-1	...	Arg	Ala	Arg	Arg	...	...	...	
60A	...	Gln	Met	Glu	Thr	...	...	...	
BMP5	...	...	...	...	...	...	...	...	
BMP6	...	Arg	...	...	...	...	...	...	
	1				5				

hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Gln	...	...	...	...	Leu	...

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		1							
	mOP-2	Ser	...	...	...	...	...	...	Leu	...
	DPP	Asp	...	Ser	...	Val	...	...	Asp	...
	Vgl	Glu	...	Lys	...	Val	...	...	...	Asn
	Vgr-1	...	...	Gln	...	Val	...	...	...	...
5	CBMP-2A	Asp	...	Ser	...	Val	...	...	Asn	...
	CBMP-2B	Asp	...	Ser	...	Val	...	...	Asn	...
	BMP3	Asp	...	Ala	...	Ile	...	...	Ser	Glu
	GDF-1	...	...	...	Glu	Val	...	...	His	Arg
	60A	Asp	...	Lys	...	...	...	...	His	...
10	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	Gln	...	...	...	...	...	...
			10					15		

	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
15	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	Val	...	...	...	Gln	...	...	Ser
	mOP-2	...	Val	...	...	...	Gln	...	...	Ser
	DPP	...	...	Val	...	...	Leu	...	...	Asp
	Vgl	...	Val	...	...	...	Gln	...	...	Met
20	Vgr-1	...	...	...	...	...	Lys	...	...	...
	CBMP-2A	...	...	Val	...	...	Pro	...	...	His
	CBMP-2B	...	...	Val	...	...	Pro	...	...	Gln
	BMP3	...	...	...	Ser	...	Lys	Ser	Phe	Asp
	GDF-1	...	Val	...	...	...	Arg	...	Phe	Leu
25	60A	...	...	...	...	...	...	...	...	Gly
	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	...	...	...	Lys	...	...	...
				20					25	

30

	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	...	...	...	...	...	...	...	Ser
	mOP-2	...	...	...	...	...	...	...	...	...
35	DPP	...	...	...	...	His	...	Lys	...	Pro

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	Vgl	...	Asn	...	...	Tyr	...	...	...	Pro
	Vgr-1	...	Asn	...	...	Asp	...	...	...	Ser
	CBMP-2A	...	Phe	...	...	His	...	Glu	...	Pro
	CBMP-2B	...	Phe	...	...	His	...	Asp	...	Pro
5	BMP3	...	...	...	...	Ser	...	Ala	...	Gln
	GDF-1	...	Asn	...	...	Gln	...	Gln	...	...
	60A	...	Phe	...	...	Ser	...	...	...	Asn
	BMP5	...	Phe	...	...	Asp	...	...	...	Ser
	BMP6	...	Asn	...	...	Asp	...	...	...	Ser
10						30				35

	hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	...	...	Asp	...	Cys	...	...	...
15	mOP-2	...	...	...	Asp	...	Cys	...	...	...
	DPP	...	...	...	Ala	Asp	His	Phe	...	Ser
	Vgl	Tyr	...	...	Thr	Glu	Ile	Leu	...	Gly
	Vgr-1	...	...	...	...	Ala	His	...	...	...
	CBMP-2A	...	...	...	Ala	Asp	His	Leu	...	Ser
20	CBMP-2B	...	...	...	Ala	Asp	His	Leu	...	Ser
	GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
	BMP3	...	...	Met	Pro	Lys	Ser	Leu	Lys	Pro
	60A	...	...	...	...	Ala	His	...	...	...
	BMP5	...	...	...	...	Ala	His	Met	...	...
25	BMP6	...	...	...	...	Ala	His	Met	...	...
						40				

	hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
	mOP-1	...	...	...	...	...	...	...	...	...
30	hOP-2	...	...	...	...	...	Leu	...	Ser	...
	mOP-2	...	...	...	...	...	Leu	...	Ser	...
	DPP	...	...	...	...	Val	...	...	...	...
	Vgl	Ser	...	...	...	...	Leu	...	...	...
	Vgr-1	...	...	...	...	...	...	...	...	...
35	CBMP-2A	...	...	...	...	...	...	...	...	...

CBMP-2B	...	...	...	...	...	...	...	...	...
BMP3	Ser	...	...	...	Thr	Ile	...	Ser	Ile
GDF-1	Leu	...	...	...	Val	Leu	Arg	Ala	...
60A	...	...	...	...	...	...	...	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
		45					50		

hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP-1	...	...	...	...	...	...	Asp	...	...
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
DPP	...	Asn	Asn	Asn	...	...	Gly	Lys	...
Vgl	...	...	Ser	...	Glu	...	...	Asp	Ile
Vgr-1	...	...	Val	Met	...	...	...	Tyr	...
CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
60A	...	...	Leu	Leu	Glu	...	Lys	Lys	...
BMP5	...	...	Leu	Met	Phe	...	Asp	His	...
BMP6	...	...	Leu	Met	...	...	...	Tyr	...
		55					60		

hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Ala	...	...	...	...	...	Lys
mOP-2	...	...	Ala	...	...	...	...	...	Lys
DPP	...	...	Ala	...	...	Val	...	...	...
Vgl	...	Leu	...	...	...	Val	...	...	Lys
Vgr-1	...	...	...	...	...	...	...	...	Lys
CBMP-2A	...	...	Ala	...	...	Val	...	...	Glu
CBMP-2B	...	...	Ala	...	...	Val	...	...	Glu

	BMP3	...	Glu	...	...	...	Val	...	Glu	Lys
	GDF-1	Asp	Leu	...	...	...	Val	...	Ala	Arg
	60A	...	...	...	...	...	...	...	...	Arg
	BMP5	...	...	...	...	...	...	...	...	Lys
5	BMP6	...	...	...	...	...	...	...	...	Lys
				65					70	

	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	mOP-1	...	...	...	...	...	...	...	...	...
10	hOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
	mOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
	Vgl	Met	Ser	Pro	...	...	Met	...	Phe	Tyr
	Vgr-1	Val	...	...	...	...	...	...	...	...
	DPP	...	Asp	Ser	Val	Ala	Met	...	...	Leu
15	CBMP-2A	...	Ser	...	...	...	Met	...	...	Leu
	CBMP-2B	...	Ser	...	...	...	Met	...	...	Leu
	BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
	GDF-1	...	Ser	Pro	...	...	...	...	Phe	...
	60A	...	Gly	...	Leu	Pro	...	...	...	His
20	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	...	...	...	...	...	...	...
					75					80

	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
25	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
	mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
	DPP	Asn	...	Gln	...	Thr	...	Val	...	...
	Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
30	Vgr-1	...	...	Asn	...	...	...	...	...	...
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
	BMP3	...	Glu	Asn	Lys	...	...	Val	...	...

GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg
60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Asn	...	...	...	...	...	...

85

hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	His	...	...	...	...	...	Lys
mOP-2	...	His	...	...	...	...	...	Lys
DPP	Asn	...	Gln	Glu	...	Thr	...	Val
Vgl	His	...	Glu	...	...	Ala	...	Asp
Vgr-1	...	...	...	...	...	...	...	...
CBMP-2A	Asn	...	Gln	Asp	...	...	...	Glu
CBMP-2B	Asn	...	Gln	Glu	...	...	...	Glu
BMP3	Val	...	Pro	...	...	Thr	...	Glu
GDF-1	Gln	...	Glu	Asp	...	...	...	Asp
60A	...	...	...	...	...	Ile	...	Lys
BMP5	...	...	...	...	...	...	...	...
BMP6	...	...	...	Trp	...	...	...	...

90   95

hOP-1	Ala	Cys	Gly	Cys	His
mOP-1	...	...	...	...	...
hOP-2	...	...	...	...	...
mOP-2	...	...	...	...	...
DPP	Gly	...	...	...	Arg
Vgl	Glu	...	...	...	Arg
Vgr-1	...	...	...	...	...
CBMP-2A	Gly	...	...	...	Arg
CBMP-2B	Gly	...	...	...	Arg
BMP3	Ser	...	Ala	...	Arg
GDF-1	Glu	...	...	...	Arg

60A	Ser	...	...	...	...
BMP5	Ser	...	...	...	...
BMP6		...	...	...	...

<div align="center">100</div>

5

**Between residues 56 and 57 of BMP3 is a Val residue;
between residues 43 and 44 of GDF-1 lies
the amino acid sequence Gly-Gly-Pro-Pro.

10

As is apparent from the foregoing amino acid
sequence comparisons, significant amino acid changes
can be made within the generic sequences while
retaining the morphogenic activity. For example, while
15 the GDF-1 protein sequence depicted in Table II shares
only about 50% amino acid identity with the hOP1
sequence described therein, the GDF-1 sequence shares
greater than 70% amino acid sequence homology (or
"similarity") with the hOP1 sequence, where "homology"
20 or "similarity" includes allowed conservative amino
acid changes within the sequence as defined by Dayoff,
et al., <u>Atlas of Protein Sequence and Structure</u> vol.5,
supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed.
Res. Fd'n, Washington D.C. 1979.)
25

The currently most preferred protein sequences
useful as morphogens in this invention include those
having greater than 60% identity, preferably greater
than 65% identity, with the amino acid sequence
30 defining the conserved six cysteine skeleton of hOP1
(e.g., residues 43-139 of Seq. ID No. 5). These most
preferred sequences include both allelic and species
variants of the OP-1 and OP-2 proteins, including the
Drosophila 60A protein. Accordingly, in still another
35 preferred aspect, the invention includes morphogens

comprising species of polypeptide chains having the
generic amino acid sequence referred to herein as
"OPX", which defines the seven cysteine skeleton and
accommodates the identities between the various
5 identified mouse and human OP1 and OP2 proteins. OPX
is presented in Seq. ID No. 29. As described therein,
each Xaa at a given position independently is selected
from the residues occurring at the corresponding
position in the C-terminal sequence of mouse or human
10 OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos.
16-23).

II.   Formulations and Methods for Administering
        Therapeutic Agents
15

     The morphogens may be provided to an individual by
any suitable means, preferably directly (e.g., locally,
as by injection to a nerve tissue locus) or
systemically (e.g., parenterally or orally). Where the
20 morphogen is to be provided parenterally, such as by
intravenous, subcutaneous, intramuscular, intraorbital,
ophthalmic, intraventricular, intracranial,
intracapsular, intraspinal, intracisternal,
intraperitoneal, buccal, rectal, vaginal, intranasal or
25 by aerosol administration, the morphogen preferably
comprises part of an aqueous solution. The solution is
physiologically acceptable so that in addition to
delivery of the desired morphogen to the patient, the
solution does not otherwise adversely affect the
30 patient's electrolyte and volume balance. The aqueous
medium for the morphogen thus may comprise normal
physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The
aqueous solution containing the morphogen can be made,
for example, by dissolving the protein in 50% ethanol
35 containing acetonitrile in 0.1% trifluoroacetic acid

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(TFA) or 0.1% HCl, or equivalent solvents. One volume
of the resultant solution then is added, for example,
to ten volumes of phosphate buffered saline (PBS),
which further may include 0.1-0.2% human serum albumin
5   (HSA). The resultant solution preferably is vortexed
extensively. If desired, a given morphogen may be made
more soluble by association with a suitable molecule.
For example, association of the mature dimer with the
pro domain of the morphogen increases solubility of the
10 protein significantly (see Section II.1, below). In
fact, the endogenous protein is thought to be
transported in this form. Another molecule capable of
enhancing solubility and particularly useful for oral
administrations, is casein. For example, addition of
15 0.2% casein increases solubility of the mature active
form of OP-1 by 80%. Other components found in milk
and/or various serum proteins also may be useful.

        Useful solutions for parenteral administration may
20 be prepared by any of the methods well known in the
pharmaceutical art, described, for example, in
Remington's Pharmaceutical Sciences (Gennaro, A., ed.),
Mack Pub., 1990. Formulations may include, for
example, polyalkylene glycols such as polyethylene
25 glycol, oils of vegetable origin, hydrogenated
naphthalenes, and the like. Formulations for direct
administration, in particular, may include glycerol and
other compositions of high viscosity. Biocompatible,
preferably bioresorbable, polymers, including, for
30 example, hyaluronic acid, collagen, polybutyrate,
tricalcium phosphate, lactide and lactide/glycolide
copolymers, may be useful excipients to control the
release of the morphogen in vivo. Other potentially
useful parenteral delivery systems for these morphogens
35 include ethylene-vinyl acetate copolymer particles,

osmotic pumps, implantable infusion systems, and
liposomes. Formulations for inhalation administration
contain as excipients, for example, lactose, or may be
aqueous solutions containing, for example,
5   polyoxyethylene-9-lauryl ether, glycocholate and
deoxycholate, or oily solutions for administration in
the form of nasal drops, or as a gel to be applied
intranasally. Formulations for parenteral
administration may also include glycocholate for buccal
10   administration, methoxysalicylate for rectal
administration, or cutric acid for vaginal
administration.

Alternatively, the morphogens described herein may
15   be administered orally. Oral administration of
proteins as therapeutics generally is not practiced as
most proteins are readily degraded by digestive enzymes
and acids in the mammalian digestive system before they
can be absorbed into the bloodstream. However, the
20   morphogens described herein typically are acid stable
and protease-resistant (see, for example, U.S. Pat.No.
4,968,590.) In addition, at least one morphogen, OP-1,
has been identified in mammary gland extract, colostrum
and 57-day milk. Moreover, the OP-1 purified from
25   mammary gland extract is morphogenically active.
Specifically, this protein induces endochondral bone
formation in mammals when implanted subcutaneously in
association with a suitable matrix material, using a
standard _in vivo_ bone assay, such as is disclosed in
30   U.S. Pat.No. 4,968,590. Moreover, the morphogen also
is detected in the bloodstream (see Example 9, below).
Finally, soluble form morphogen, e.g., mature morphogen
associated with the pro domain, is capable of
maintaining neural pathways in a mammal (See Examples 4
35   and 6 below). These findings indicate that oral and

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parenteral administration are viable means for
administering morphogens to an individual. In
addition, while the mature forms of certain morphogens
described herein typically are sparingly soluble, the
5   morphogen form found in milk (and mammary gland extract
and colostrum) is readily soluble, probably by
association of the mature, morphogenically active form
with part or all of the pro domain of the intact
sequence and/or by association with one or more milk
10   components. Accordingly, the compounds provided herein
also may be associated with molecules capable of
enhancing their solubility _in vitro_ or _in vivo_.

The compounds provided herein also may be
15   associated with molecules capable of targeting the
morphogen or morphogen-stimulating agent to nerve
tissue. For example, an antibody, antibody fragment,
or other binding protein that interacts specifically
with a surface molecule on nerve tissue cells,
20   including neuronal or glial cells, may be used. Useful
targeting molecules may be designed, for example, using
the single chain binding site technology disclosed, for
example, in U.S. Pat. No. 5,091,513.

25   As described above, the morphogens provided herein
share significant sequence homology in the C-terminal
active domains. By contrast, the sequences typically
diverge significantly in the sequences which define the
pro domain. Accordingly, the pro domain is thought to
30   be morphogen-specific. As described above, it is also
known that the various morphogens identified to date
are differentially expressed in the different tissues.
Accordingly, without being limited to any given theory,
it is likely that, under natural conditions in the
35   body, selected morphogens typically act on a given

tissue. Accordingly, part or all of the pro domains
which have been identified associated with the active
form of the morphogen in solution, may serve as
targeting molecules for the morphogens described

5 herein. For example, the pro domains may interact
specifically with one or more molecules at the target
tissue to direct the morphogen associated with the pro
domain to that tissue. Accordingly, another useful
targeting molecule for targeting morphogen to nerve

10 tissue is part or all of a morphogen pro domain,
particularly part or all of the pro domains of OP-1 or
GDF-1, both of which proteins are found naturally
associated with nerve tissue.

15 Finally, the morphogens or morphogen-stimulating
agents provided herein may be administered alone or in
combination with other molecules known to be beneficial
in maintaining neural pathways, including nerve growth
factors and anti-inflammatory agents.

20

The compounds provided herein can be formulated
into pharmaceutical compositions by admixture with
pharmaceutically acceptable nontoxic excipients and
carriers. As noted above, such compositions may be

25 prepared for parenteral administration, particularly in
the form of liquid solutions or suspensions; for oral
administration, particularly in the form of tablets or
capsules; or intranasally, particularly in the form of
powders, nasal drops, or aerosols.

30

The compositions can be formulated for parenteral
or oral administration to humans or other mammals in
therapeutically effective amounts, e.g., amounts which
provide appropriate concentrations for a time

35 sufficient to eliminate or reduce the patient's

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pathological condition, to provide therapy for the
neurological diseases and disorders described above,
and amounts effective to enhance neural cell survival
an/or to protect neurons and neural pathways in
5   anticipation of injury to nerve tissue.

As will be appreciated by those skilled in the art,
the concentration of the compounds described in a
therapeutic composition will vary depending upon a
10 number of factors, including the dosage of the drug to
be administered, the chemical characteristics (e.g.,
hydrophobicity) of the compounds employed, and the
route of administration. The preferred dosage of drug
to be administered also is likely to depend on such
15 variables as the type and extent of progression of the
neurological disease, the overall health status of the
particular patient, the relative biological efficacy of
the compound selected, the formulation of the compound
excipients, and its route of administration. In general
20 terms, the compounds of this invention may be provided
in an aqueous physiological buffer solution containing
about 0.1 to 10% w/v compound for parenteral
administration. Typical dose ranges are from about 10
ng/kg to about 1 g/kg of body weight per day; a
25 preferred dose range is from about 0.1 μg/kg to
100 mg/kg of body weight per day. Optimally, the
morphogen dosage given in all cases is between 2-20 μg
of protein per kilogram weight of the patient per day.
No obvious OP-1 induced pathological lesions are
30 induced when mature morphogen (e.g., OP-1, 20 μg) is
administered daily to normal growing rats for

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21 consecutive days. Moreover, 10 μg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalties.

5

Since the ability of proteins and protein fragments to penetrate the blood-brain barrier may be related to their size, lipophilicity or their net ionic charge, suitable modifications of the morphogens may be

10   formulated (e.g., by substituting pentafluorophenylalanine for phenylalanine, or by conjugation to a cationized protein such as albumin) to increase their transportability across the barrier, using standard methodologies known in the art. See,

15   for example, Kastin et al., Pharmac. Biochem. Behav. (1979) 11:713-716; Rapoport et al., Science (1980) 207:84-86; Pardridge et al., (1987) Biochem. Biophys. Res. Commun. 146:307-313; Riekkinen et al.,(1987) Peptides 8:261-265. The efficacy of these functional

20   analogs may be assessed for example, by evaluating the ability of these compounds to induce redifferentiation of transformed cells, or enhance survival of neurons at risk of dying, as described in the Examples provided herein.

25

In administering morphogens systemically in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a

30   maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

Where injury to neurons of a neural pathway is
induced deliberately as part of, for example, a
surgical procedure, the morphogen preferably is
provided just prior to, or concomitant with induction
5   of the trauma. Preferably, the morphogen is
administered prophylactically in a surgical setting.
Optimally, the morphogen dosage given in all cases is
between 2-20 µg of protein per kilogram weight of the
patient.
10

Alternatively, an effective amount of an agent
capable of stimulating endogenous morphogen levels may
be administered by any of the routes described above.
For example, an agent capable of stimulating morphogen
15   production and/or secretion from nerve tissue cells may
be provided to a mammal, e.g., by direct administration
of the morphogen to glial cells associated with the
nerve tissue to be treated. A method for identifying
and testing agents capable of modulating the levels of
20   endogenous morphogens in a given tissue is described
generally herein in Example 13, and in detail in
internatinal application US92/07359 (WO93/015172), the
disclosure of which is incorporated herein by
reference. Briefly, candidate compounds can be
25   identified and tested by incubating the compound in
vitro with a test tissue or cells thereof, for a time
sufficient to allow the compound to affect the
production, i.e., the expression and/or secretion, of a
morphogen produced by the cells of that tissue.   Here,
30   suitable tissue or cultured cells of a tissue
preferably would comprise neurons and/or glial cells.
For example, suitable tissue for testing may include
cultured cells isolated from the substantia nigra,
adendema glial cells, and the like.
35

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A currently preferred detection means for evaluating the level of the morphogen in culture upon exposure to the candidate compound comprises an immunoassay utilizing an antibody or other suitable
5 binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific for that
10 morphogen. As described herein, morphogens may be isolated from natural-sourced material or they may be recombinantly produced. Agents capable of stimulating endogenous morphogens then may formulated into pharmaceutical preparations and administered as
15 described herein.

Where the morphogen is to be provided to a site to stimulate axon regeneration, the morphogen preferably is provided to the site in association with a
20 biocompatible, preferably bioresorbable carrier suitable for maintaining a protein at a site _in_ _vivo_, and through which a neural process may regenerate. A currently preferred carrier also comprises sufficient structure to assist direction of axonal growth.
25 Currently preferred carriers include structural molecules such as collagen, hyaluronic acid or laminin, and/or synthetic polymers or copolymers of, for example, polylactic acid, polyglycolic acid or polybutyric acid. Currently most preferred are
30 carriers comprising tissue extracellular matrix. These may be obtained commercially. In addition, a brain

tissue-derived extracellular matrix may be prepared as described in international application US92/01968 (WO92/15323), incorporated hereinabove by reference, and/or by other means known in the art.

5

The currently preferred means for repairing breaks in neural pathways, particularly pathways of the peripheral nervous system, include providing the morphogen to the site as part of a device that includes

10 a biocompatible membrane or casing of a dimension sufficient to span the break and having openings adapted to receive severed nerve ends. The morphogen is disposed within the casing, preferably dispersed throughout a suitable carrier, and is accessible to the

15 severed nerve ends. Alternatively, the morphogen may be adsorbed onto the interior surface of the casing, or otherwise associated therewith. In addition, currently preferred casings have an impermeable exterior surface. The casing acts as a nerve guidance channel, aiding in

20 directing axonal growth. In addition, the casing also protects the damaged nerve from immunologically-related agents which may assist in scar tissue formation. Suitable channel or casing materials include silicone or bioresorbable materials such as collagen, hyaluronic

25 acid, laminin, polylactic acid, polyglycolic acid, polybutyric acid and the like. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may

30 be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section.

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Moreover the guidance channels may be constructed of
two or more parts which may be clamped together to
secure the nerve stumps. Nerve endings may be secured
to the nerve guidance channels by means of sutures,
5   biocompatible adhesives such as fibrin glue, or other
means known in the medical art.

II.1   Soluble Morphogen Complexes

10   A currently preferred form of the morphogen useful
in therapeutic formulations, having improved solubility
in aqueous solutions and consisting essentially of
amino acids, is a dimeric morphogenic protein
comprising at least the 100 amino acid peptide sequence
15   having the pattern of seven or more cysteine residues
characteristic of the morphogen family complexed with a
peptide comprising part or all of a pro region of a
member of the morphogen family, or an allelic, species
or other sequence variant thereof. Preferably, the
20   dimeric morphogenic protein is complexed with two
peptides. Also, the dimeric morphogenic protein
preferably is noncovalently complexed with the pro
region peptide or peptides. The pro region peptides
also preferably comprise at least the N-terminal
25   eighteen amino acids that define a given morphogen
pro region. In a most preferred embodiment, peptides
defining substantially the full length pro region are
used.

30   Other soluble forms of morphogens include dimers of
the uncleaved pro forms of these proteins, as well as
"hemi-dimers" wherein one subunit of the dimer is an
uncleaved pro form of the protein, and the other

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subunit comprises the mature form of the protein,
including truncated forms thereof, preferably
noncovalently associated with a cleaved pro domain
peptide.

5

As described above, useful pro domains include the
full length pro regions, as well as various truncated
forms hereof, particularly truncated forms cleaved at
proteolytic Arg-Xaa-Xaa-Arg cleavage sites. For

10   example, in OP-1, possible pro sequences include
sequences defined by residues 30-292 (full length
form); 48-292; and 158-292. Soluble OP-1 complex
stability is enhanced when the pro region comprises the
full length form rather than a truncated form, such as

15   the 48-292 truncated form, in that residues 30-47 show
sequence homology to the N-terminal portions of other
morphogens, and are believed to have particular utility
in enhancing complex stability for all morphogens.
Accordingly, currently preferred pro sequences are

20   those encoding the full length form of the pro region
for a given morphogen. Other pro sequences
contemplated to have utility include biosynthetic pro
sequences, particularly those that incorporate a
sequence derived from the N-terminal portion of one or

25   more morphogen pro sequences.

As will be appreciated by those having ordinary
skill in the art, useful sequences encoding the pro
region may be obtained from genetic sequences encoding

30   known morphogens. Alternatively, chimeric pro regions
can be constructed from the sequences of one or more
known morphogens. Still another option is to create a
synthetic sequence variant of one or more known pro
region sequences.

35

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In another preferred aspect, useful pro region
peptides include polypeptide chains comprising an amino
acid sequence encoded by a nucleic acid that hybridizes
under stringent conditions with a DNA or RNA sequence
5 encoding at least the N-terminal eighteen amino acids
of the pro region sequence for OP1 or OP2, e.g.,
nucleotides 136-192 and 152-211 of Seq. ID No. 16 and
20, respectively.

10 A.   Isolation of Soluble morphogen complex from
          conditioned media or body fluid

Morphogens are expressed from mammalian cells as
soluble complexes. Typically, however the complex is
15 disassociated during purification, generally by
exposure to denaturants often added to the purification
solutions, such as detergents, alcohols, organic
solvents, chaotropic agents and compounds added to
reduce the pH of the solution. Provided below is a
20 currently preferred protocol for purifying the soluble
proteins from conditioned media (or, optionally, a body
fluid such as serum, cerebro-spinal or peritoneal
fluid), under non-denaturing conditions. The method is
rapid, reproducible and yields isolated soluble
25 morphogen complexes in substantially pure form.

Soluble morphogen complexes can be isolated from
conditioned media using a simple, three step
chromatographic protocol performed in the absence of
30 denaturants. The protocol involves running the media
(or body fluid) over an affinity column, followed by
ion exchange and gel filtration chromatographies. The
affinity column described below is a Zn-IMAC column.
The present protocol has general applicability to the
35 purification of a variety of morphogens, all of which

are anticipated to be isolatable using only minor
modifications of the protocol described below. An
alternative protocol also envisioned to have utility an
immunoaffinity column, created using standard
5 procedures and, for example, using antibody specific
for a given morphogen pro domain (complexed, for
example, to a protein A-conjugated Sepharose column.)
Protocols for developing immunoaffinity columns are
well described in the art, (see, for example, <u>Guide to</u>
10 <u>Protein Purification</u>, M. Deutscher, ed., Academic
Press, San Diego, 1990, particularly sections VII and
XI.)

   In this experiment OP-1 was expressed in mammalian
15 CHO (chinese hamster ovary) cells as described in the
art (see, for example, international application
US90/05903 (WO91/05802).) The CHO cell conditioned
media containing 0.5% FBS was initially purified using
Immobilized Metal-Ion Affinity Chromatography (IMAC).
20 The soluble OP-1 complex from conditioned media binds
very selectively to the Zn-IMAC resin and a high
concentration of imidazole (50 mM imidazole, pH 8.0) is
required for the effective elution of the bound
complex. The Zn-IMAC step separates the soluble OP-1
25 from the bulk of the contaminating serum proteins that
elute in the flow through and 35 mM imidazole wash
fractions. The Zn-IMAC purified soluble OP-1 is next
applied to an S-Sepharose cation-exchange column
equilibrated in 20 mM $NaPO_4$ (pH 7.0) with 50 mM NaCl.
30 This S-Sepharose step serves to further purify and
concentrate the soluble OP-1 complex in preparation for
the following gel filtration step. The protein was

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applied to a Sephacryl S-200HR column equilibrated in
TBS. Using substantially the same protocol, soluble
morphogens also may be isolated from one or more body
fluids, including serum, cerebro-spinal fluid or
5 peritoneal fluid.

   IMAC was performed using Chelating-Sepharose
(Pharmacia) that had been charged with three column
volumes of 0.2 M $ZnSO_4$. The conditioned media was
10 titrated to pH 7.0 and applied directly to the ZN-IMAC
resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM
NaCl. The Zn-IMAC resin was loaded with 80 mL of
starting conditioned media per mL of resin. After
loading, the column was washed with equilibration
15 buffer and most of the contaminating proteins were
eluted with 35 mM imidazole (pH 7.0) in equilibration
buffer. The soluble OP-1 complex then is eluted with
50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM
NaCl.
20

   The 50 mM imidazole eluate containing the soluble
OP-1 complex was diluted with nine volumes of 20 mM
$NaPO_4$ (pH 7.0) and applied to an S-Sepharose
(Pharmacia) column equilibrated in 20 mM $NaPO_4$ (pH 7.0)
25 with 50 mM NaCl. The S-Sepharose resin was loaded with
an equivalent of 800 mL of starting conditioned media
per mL of resin. After loading the S-Sepharose column
was washed with equilibration buffer and eluted with
100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM
30 $NaPO_4$ (pH 7.0). The 300 mM NaCl pool was further
purified using gel filtration chromatography. Fifty
mls of the 300 mm NaCl eluate was applied to a 5.0 X 90
cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris
buffered saline (TBS), 50 mM Tris, 150 mM NaCl
35 (pH 7.4). The column was eluted at a flow rate of 5

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mL/minute collecting 10 mL fractions. The apparent
molecular of the soluble OP-1 was determined by
comparison to protein molecular weight standards
(alcohol dehydrogenase (ADH, 150 kDa), bovine serum
5   albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa)
and cytochrome C (cyt C, 12.5 kDa). The purity of the
S-200 column fractions was determined by separation on
standard 15% polyacrylamide SDS gels stained with
coomassie blue. The identity of the mature OP-1 and
10   the pro-domain was determined by N-terminal sequence
analysis after separation of the mature OP-1 from the
pro-domain using standard reverse phase C18 HPLC.

The soluble OP-1 complex elutes with an apparent
15   molecular weight of 110 kDa. This agrees well with the
predicted composition of the soluble OP-1 complex with
one mature OP-1 dimer (35-36 kDa) associated with two
pro-domains (39 kDa each). Purity of the final complex
can be verified by running the appropriate fraction in
20   a reduced 15% polyacrylamide gel.

The complex components can be verified by running
the complex-containing fraction from the S-200 or S-
200HR columns over a reverse phase C18 HPLC column and
25   eluting in an acetonitrile gradient (in 0.1% TFA),
using standard procedures. The complex is dissociated
by this step, and the pro domain and mature species
elute as separate species. These separate species then
can be subjected to N-terminal sequencing using
30   standard procedures (see, for example, Guide to
Protein Purification, M. Deutscher, ed., Academic
Press, San Diego, 1990, particularly pp. 602-613), and
the identity of the isolated 36kD, 39kDa proteins
confirmed as mature morphogen and isolated, cleaved pro
35   domain, respectively. N-terminal sequencing of the

isolated pro domain from mammalian cell produced OP-1
revealed 2 forms of the pro region, the intact form
(beginning at residue 30 of Seq. ID No. 16) and a
truncated form, (beginning at residue 48 of Seq. ID No.
5   16.) N-terminal sequencing of the polypeptide subunit
of the isolated mature species reveals a range of
N-termini for the mature sequence, beginning at
residues 293, 300, 313, 315, 316, and 318, of Seq. ID
No. 16, all of which are active as demonstrated by the
10 standard bone induction assay.

B.   In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes
15 from culture media or a body fluid, soluble complexes
may be formulated from purified pro domains and mature
dimeric species. Successful complex formation
apparently requires association of the components under
denaturing conditions sufficient to relax the folded
20 structure of these molecules, without affecting
disulfide bonds. Preferably, the denaturing conditions
mimic the environment of an intracellular vesicle
sufficiently such that the cleaved pro domain has an
opportunity to associate with the mature dimeric
25 species under relaxed folding conditions. The
concentration of denaturant in the solution then is
decreased in a controlled, preferably step-wise manner,
so as to allow proper refolding of the dimer and pro
regions while maintaining the association of the pro
30 domain with the dimer. Useful denaturants include 4-6M
urea or guanidine hydrochloride (GuHCl), in buffered
solutions of pH 4-10, preferably pH 6-8. The soluble
complex then is formed by controlled dialysis or
dilution into a solution having a final denaturant
35 concentration of less than 0.1-2M urea or GuHCl,

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preferably 1-2 M urea of GuHCl, which then preferably
can be diluted into a physiological buffer. Protein
purification/renaturing procedures and considerations
are well described in the art, and details for
5   developing a suitable renaturing protocol readily can
be determined by one having ordinary skill in the art.
One useful text one the subject is <u>Guide to Protein
Purification</u>, M. Deutscher, ed., Academic Press, San
Diego, 1990, particularly section V. Complex formation
10 also may be aided by addition of one or more chaperone
proteins.

C.   <u>Stability of Soluble Morphogen Complexes</u>

15   The stability of the highly purified soluble
morphogen complex in a physiological buffer, e.g.,
tris-buffered saline (TBS) and phosphate-buffered
saline (PBS), can be enhanced by any of a number of
means. Currently preferred is by means of a pro region
20 that comprises at least the first 18 amino acids of the
pro sequence (e.g., residues 30-47 of Seq. ID NO. 16
for OP-1), and preferably is the full length pro
region. Residues 30-47 show sequence homology to the
N-terminal portion of other morphogens and are believed
25 to have particular utility in enhancing complex
stability for all morphogens. Other useful means for
enhancing the stability of soluble morphogen complexes
include three classes of additives. These additives
include basic amino acids (e.g., L-arginine, lysine and
30 betaine); nonionic detergents (e.g., Tween 80 or
NonIdet P-120); and carrier proteins (e.g., serum

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albumin and casein). Useful concentrations of these
additives include 1-100 mM, preferably 10-70 mM,
including 50 mM, basic amino acid;, 0.01-1.0%,
preferably 0.05-0.2%, including 0.1% (v/v) nonionic
5   detergent;, and 0.01-1.0%, preferably 0.05-0.2%,
including 0.1% (w/v) carrier protein.

III.   <u>Examples</u>

10   Example 1.   <u>Identification of Morphogen-Expressing</u>
        <u>Tissue</u>

        Determining the tissue distribution of morphogens
     may be used to identify different morphogens expressed
15   in a given tissue, as well as to identify new, related
     morphogens. Tissue distribution also may be used to
     identify useful morphogen-producing tissue for use in
     screening and identifying candidate morphogen-
     stimulating agents. The morphogens (or their mRNA
20   transcripts) readily are identified in different
     tissues using standard methodologies and minor
     modifications thereof in tissues where expression may
     be low. For example, protein distribution may be
     determined using standard Western blot analysis or
25   immunofluorescent techniques, and antibodies specific
     to the morphogen or morphogens of interest. Similarly,
     the distribution of morphogen transcripts may be
     determined using standard Northern hybridization
     protocols and transcript-specific probes.
30
        Any probe capable of hybridizing specifically to a
     transcript, and distinguishing the transcript of
     interest from other, related transcripts may be used.
     Because the morphogens described herein share such high
35   sequence homology in their active, C-terminal domains,

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the tissue distribution of a specific morphogen
transcript may best be determined using a probe
specific for the pro region of the immature protein
and/or the N-terminal region of the mature protein.

5   Another useful sequence is the 3' non-coding region
flanking and immediately following the stop codon.
These portions of the sequence vary substantially among
the morphogens of this invention, and accordingly, are
specific for each protein. For example, a particularly

10   useful Vgr-1-specific probe sequence is the PvuII-SacI
fragment, a 265 bp fragment encoding both a portion of
the untranslated pro region and the N-terminus of the
mature sequence (see Lyons et al. (1989) PNAS 86:4554-
4558 for a description of the cDNA sequence).

15   Similarly, particularly useful mOP-1-specific probe
sequences are the BstX1-BglI fragment, a 0.68 Kb
sequence that covers approximately two-thirds of the
mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb
sequence immediately upstream of the 7-cysteine domain;

20   and the Ear1-Pst1 fragment, an 0.3 Kb fragment
containing a portion of the 3'untranslated sequence
(See Seq. ID No. 18, where the pro region is defined
essentially by residues 30-291.) Similar approaches
may be used, for example, with hOP-1 (Seq. ID No. 16)

25   or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which
may be synthetically engineered or obtained from cloned
sequences, morphogen transcripts can be identified in

30   mammalian tissue, using standard methodologies well
known to those having ordinary skill in the art.
Briefly, total RNA is prepared from various adult
murine tissues (e.g., liver, kidney, testis, heart,
brain, thymus and stomach) by a standard methodology

35   such as by the method of Chomczyaski et al. ((1987)

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Anal. Biochem 162:156-159) and described below. Poly
(A)+ RNA is prepared by using oligo (dT)-cellulose
chromatography (e.g., Type 7, from Pharmacia LKB
Biotechnology, Inc.).   Poly (A)+ RNA (generally 15 μg)
5   from each tissue is fractionated on a 1%
agarose/formaldehyde gel and transferred onto a Nytran
membrane (Schleicher & Schuell). Following the
transfer, the membrane is baked at 80°C and the RNA is
cross-linked under UV light (generally 30 seconds at 1
10   mW/cm^2). Prior to hybridization, the appropriate probe
is denatured by heating. The hybridization is carried
out in a lucite cylinder rotating in a roller bottle
apparatus at approximately 1 rev/min for approximately
15 hours at 37°C using a hybridization mix of 40%
15   formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS.
Following hybridization, the non-specific counts are
washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of
20   various morphogens, including Vgr-1, OP-1, BMP2, BMP3,
BMP4, BMP5, GDF-1, and OP-2 in developing and adult
tissue are disclosed in international application
US92/01968 (WO92/15323), and in Ozkaynak, et al.,
(1991) Biochem. Biophys. Res. Commn. 179:116-123, and
25   Ozkaynak, et al. (1992) J. Biol.Chem. 267: 25220-25227.
Using the general probing methodology described herein,
northern blot hybridizations using probes specific for
these morphogens to probe brain, spleen, lung, heart,
liver and kidney tissue indicate that kidney-related
30   tissue appears to be the primary expression source for
OP-1, with brain, heart and lung tissues being
secondary sources. Lung tissue appears to be the
primary tissue expression source for Vgr-1, BMP5, BMP4
and BMP3. Lower levels of Vgr-1 also are seen in kidney
35   and heart tissue, while the liver appears to be a

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seen in kidney and heart tissue, while the liver
appears to be a secondary expression source for BMP5,
and the spleen appears to be a secondary expression
source for BMP4. GDF-1 appears to be expressed
5   primarily in brain tissue. To date, OP-2 appears to be
expressed primarily in early embryonic tissue.
Specifically, northern blots of murine embryos and 6-
day post-natal animals shows abundant OP2 expression in
8-day embryos. Expression is reduced significantly in
10   17-day embryos and is not detected in post-natal
animals.

Example 2.   Morphogen Localization in the Nervous
             System
15

Morphogens have been identified in developing and
adult rat brain and spinal cord tissue, as determined
both by northern blot hybridization of morphogen-
specific probes to mRNA extracts from developing and
20   adult nerve tissue (see Example 1, above) and by
immunolocalization studies. For example, northern blot
analysis of developing rat tissue has identified
significant OP-1 mRNA transcript expression in the CNS
international application US92/01968 (WO92/15323), and
25   Ozkaynak et al. (1991) Biochem. Biophys. Res. Comm.,
179:11623 and Ozkaynak, et al. (1992) J. Biol. Chem.
267:25220-25227. GDF-1 mRNA appears to be expressed
primarily in developing and adult nerve tissue,
specifically in the brain, including the cerebellum and
30   brain stem, spinal cord and peripheral nerves (Lee, S.
(1991) PNAS 88: 4250-4254). BMP2B (also referred in
the art as BMP4) and Vgr-1 transcripts also have been
reported to be expressed in nerve tissue (Jones et al.
(1991) Development 111:531-542), although the nerve
35   tissue does not appear to be the primary expression

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tissue for these genes (Ozkaynak, et al., (1992) J.
Biol. Chem. 267:25220-25227. Specifically, CBMP2
transcripts are reported in the region of the
diencephalon associated with pituitary development, and
5   Vgr-1 transcripts are reported in the anteroposterior
axis of the CNS, including in the roof plate of the
developing neural tube, as well as in the cells
immediately adjacent the floor plate of the developing
neural tube. In older rats, Vgr-1 transcripts are
10 reported in developing hippocampus tissue. In
addition, the genes encoding OP-1 and BMP2 originally
were identified by probing human hippocampus cDNA
libraries.

15   Immunolocalization studies, performed using
standard methodologies known in the art and disclosed
in international application US92/01968 (WO92/15323),
the disclosure of which is incorporated herein,
localized OP-1 expression to particular areas of
20 developing and adult rat brain and spinal cord tissue.
Specifically, OP-1 protein expression was assessed in
adult (2-3 months old) and five or six-day old mouse
embryonic nerve tissue, using standard morphogen-
specific antisera, specifically, rabbit anti-OP1
25 antisera, made using standard antibody protocols known
in the art and preferably purified on an OP-1 affinity
column. The antibody itself was labelled using
standard fluorescent labelling techniques, or a
labelled anti-rabbit IgG molecule was used to visualize
30 bound OP-1 antibody.

As can be seen in FIG 1A and 1B, immunofluorescence
staining demonstrates the presence of OP-1 in adult rat
central nervous system (CNS.) Similar and extensive
35 staining is seen in both the brain (1A) and spinal cord

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(1B). OP-1 appears to be localized predominantly to
the extracellular matrix of the grey matter (neuronal
cell bodies), distinctly present in all areas except
the cell bodies themselves. In white matter, formed
5 mainly of myelinated nerve fibers, staining appears to
be confined to astrocytes (glial cells). A similar
staining pattern also was seen in newborn rat (10 day
old) brain sections.

10   In addition, OP-1 has been specifically localized
in the substantia nigra, which is composed primarily of
striatal basal ganglia, a system of accessory motor
neurons that function is association with the cerebral
cortex and corticospinal system. Dysfunctions in this
15 subpopulation or system of neurons are associated with
a number of neuropathies, including Huntington's chorea
and Parkinson's disease.

OP1 also has been localized at adendema glial
20 cells, known to secrete factors into the cerebrospinal
fluid, and which occur around the intraventricular
valve, coroid fissure, and central canal of the brain
in both developing and adult rat.

25   Finally, morphogen inhibition in developing embryos
inhibits nerve tissue development. Specifically, 9-day
mouse embryo cells, cultured in vitro under standard
culturing conditions, were incubated in the presence
and absence of an OP-1-specific monoclonal antibody
30 prepared using recombinantly produced, purified mature
OP-1 and the immunogen. The antibody was prepared
using standard antibody production means well known in
the art and as described generally in Example 13.
After two days, the effect of the antibody on the
35 developing embryo was evaluated by histology. As

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determined by histological examination, the OP-1-
specific antibody specifically inhibits eye lobe
formation in the developing embryo. In particular, the
diencephalon outgrowth does not develop. In addition,
5   the heart is malformed and enlarged. Moreover, in
separate immunolocalization studies on embryo sections
with labelled OP-1 specific antibody, the OP-1-specific
antibody localizes to neural epithelia.

10   The endogenous morphogens which act on neuronal
cells may be expressed and secreted by nerve tissue
cells, e.g., by neurons and/or glial cells associated
with the neurons, and/or they may be transported to the
neurons by the cerebrospinal fluid and/or bloodstream.
15 Recently, OP-1 has been identified in the human blood
(See Example 9, below). In addition, transplanted
Schwann cells recently have been shown to stimulate
nerve fiber formation in rat spinal cord, including
inducing vascularization and myelin sheath formation
20 around at least some of the new neuronal processes
(Bunge (1991) Exp. Neurology 114:254-257.) The
regenerative property of these cells may be mediated by
the secretion of a morphogen by the Schwann cells.

25 Example 3.   Morphogen Enhancement of Neuronal Cell
                    Survival

        The morphogens described herein enhance cell
survival, particularly of neuronal cells at risk of
30 dying. For example, fully differentiated neurons are
non-mitotic and die in vitro when cultured under
standard mammalian cell culture conditions, using a
chemically defined or low serum medium known in the
art, (see, for example, Charness (1986) J. Biol. Chem.
35 26:3164-3169 and Freese et al. (1990) Brain Res.

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<u>521</u>:254-264.) However, if a primary culture of non-
mitotic neuronal cells is treated with a morphogen, the
survival of these cells is enhanced significantly. For
example, a primary culture of striatal basal ganglia
5   isolated from the substantia nigra of adult rat brain
was prepared using standard procedures, e.g., by
dissociation by trituration with pasteur pipette of
substania nigra tissue, using standard tissue culturing
protocols, and grown in a low serum medium, e.g.,
10   containing 50% DMEM (Dulbecco's modified Eagle's
medium), 50% F-12 medium, heat inactivated horse serum
supplemented with penicillin/streptomycin and 4 g/l
glucose. Under standard culture conditions, these
cells are undergoing significant cell death by three
15   weeks when cultured in a serum-free medium. Cell death
is evidenced morphologically by the inability of cells
to remain adherent and by changes in their
ultrastructural characteristics, e.g., by chromatin
clumping and organelle disintegration.
20

In this example, the cultured basal ganglia were
were treated with chemically defined medium conditioned
with 0.1-100 ng/ml OP-1. Fresh, morphogen-conditioned
medium was provided to the cells every 3-4 days. Cell
25   survival was enhanced significantly and was dose
dependent upon the level of OP-1 added: cell death
decreased significantly as the concentration of OP-1
was increased in cell cultures. Specifically, cells
remained adherent and continued to maintain the
30   morphology of viable differentiated neurons. In the
absence of morphogen treatment, the majority of the
cultured cells dissociated and underwent cell necrosis.

Dysfunctions in the basal ganglia of the sustantia nigra are associated with Huntington's chorea and parkinsonism _in_ _vivo_. The ability of the morphogens defined herein to enhance neuron survival indicates

5 that these morphogens will be useful as part of a therapy to enhance survival of neuronal cells at risk of dying _in_ _vivo_ due, for example, to a neuropathy or chemical or mechanical trauma. It is particularly anticipated that these morphogens will provide a useful

10 therapeutic agent to treat neuropathies which affect the striatal basal ganglia, including Huntington's chorea and Parkinson's disease. For clinical applications, the morphogen may be administered or, alternatively, a morphogen-stimulating agent may be

15 administered.

Example 4.   Morphogen-Induced Redifferentiation of Transformed Cells

20

The morphogens described herein also induce redifferentiation of transformed cells to a morphology characteristic of untransformed cells. In particular, the morphogens are capable of inducing

25 redifferentiation of transformed cells of neuronal origin to a morphology characteristic of untransformed neurons. The example provided below details morphogen induced redifferentiation of a transformed human cell line of neuronal origin, NG105-115. Morphogen-induced

30 redifferentiation of transformed cells also has been shown in mouse neuroblastoma cells (N1E-115) and in human embryo carcimona cells (see international application US92/01968 (WO92/15323).

NG108-15 is a transformed hybrid cell line produced
by fusing neuroblastoma x glioma cells (obtained from
America Type Tissue Culture, Rockville, MD), and
exhibiting a morphology characteristic of transformed
5   embryonic neurons, e.g., having a fibroblastic
morphology. Specifically, the cells have polygonal
cell bodies, short, spike-like processes and make few
contacts with neighboring cells (see FIG. 1A).
Incubation of NG108-15 cells, cultured in a chemically
10   defined, serum-free medium, with 0.1 to 300 ng/ml of
OP-1 for four hours induces an orderly, dose-dependent
change in cell morphology.

In the experiment NG108-15 cells were subcultured
15   on poly-L-lysine coated 6-well plates. Each well
contained 40-50,000 cells in 2.5 ml of chemically
defined medium. On the third day 2.5 µl of OP-1 in 60%
ethanol containing 0.025% trifluoroacetic was added to
each well. OP-1 concentrations of 0-300 ng/ml were
20   tested. Typically, the media was changed daily with new
aliquots of OP-1, although morphogenesis can be induced
by a single four hour incubation with OP-1. In
addition, OP-1 concentrations of 10 ng/ml were
sufficient to induce redifferentiation. After one day,
25   hOP-1-treated cells undergo a significant change in
their cellular ultrastructure, including rounding of
the soma, increase in phase brightness and extension of
the short neurite processes. After two days, cells
treated with OP-1 begin to form epithelioid sheets,
30   which provide the basis for the growth of mutilayered
aggregates at three day's post-treatment. By four
days, the great majority of OP-1-treated cells are
associated in tightly-packed, mutilayered aggregates

(Fig. 1B). Fig. 2 plots the mean number of multi-
layered aggregates or cell clumps identified in twenty
randomly selected fields from six independent
experiments, as a function of morphogen concentration.
5   Forty ng/ml of OP-1 is sufficient for half maximum
induction of cell aggregation.

The morphogen-induced redifferentiation occurred
without any associated changes in DNA synthesis, cell
10 division, or cell viability, making it unlikely that
the morphologic changes were secondary to cell
differentiation or a toxic effect of hOP-1. Moreover,
the OP-1-induced morphogenesis does not inhibit cell
division, as determined by ^{3}H-thymidine uptake, unlike
15 other molecules which have been shown to stimulate
differentiation of transformed cells, such as butyrate,
DMSO, retanoic acid or Forskolin. The data indicate
that OP-1 can maintain cell stability and viability
after inducing redifferentiation. In addition, the
20 effects are morphogen specific, and redifferentiation
is not induced when NG108-15 cells are incubated with
0.1-40 ng/ml TGF-β.

The experiments also have been performed with
25 highly purified soluble morphogen (e.g., mature OP1
associated with its pro domain) which also specifically
induced redifferentiation of NG108-15 cells.

The morphogens described herein accordingly provide
30 useful therapeutic agents for the treatment of
neoplasias and neoplastic lesions of the nervous
system, particularly in the treatment of

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neuroblastomas, including retinoblastomas, and gliomas.
The morphogens themselves may be administered or,
alternatively, a morphogen-stimulating agent may be
administered.

Example 5.   Nerve Tissue Protection from Chemical
             Trauma

The ability of the morphogens described herein to
enhance survival of neuronal cells and to induce cell
aggregation and cell-cell adhesion in redifferentiated
cells, indicates that the morphogens will be useful as
therapeutic agents to maintain neural pathways by
protecting the cells defining the pathway from the
damage caused by chemical trauma. In particular, the
morphogens can protect neurons, including developing
neurons, from the effects of toxins known to inhibit
the proliferation and migration of neurons and to
interfere with cell-cell adhesion. Examples of such
toxins include ethanol, one or more of the toxins
present in cigarette smoke, and a variety of opiates.
The toxic effects of ethanol on developing neurons
induces the neurological damage manifested in fetal
alcohol syndrome. The morphogens also may protect
neurons from the cytoxic effects associated with
excitatory amino acids such as glutamate.

For example, ethanol inhibits the cell-cell
adhesion effects induced in morphogen-treated NG108-15
cells when provided to these cells at a concentration
of 25-50 mM. Half maximal inhibition can be achieved
with 5-10 mM ethanol, the concentration of blood
alcohol in an adult following ingestion of a single
alcoholic beverage. Ethanol likely interferes with the

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homophilic binding of CAMs between cells, rather than
their induction, as morphogen-induced N-CAM levels are
unaffected by ethanol. Moreover, the inhibitory effect
is inversely proportional to morphogen concentration.
5   Accordingly, it is envisioned that administration of a
morphogen or morphogen-stimulating agent to neurons,
particularly developing neurons, at risk of damage from
exposure to toxins such as ethanol, may protect these
cells from nerve tissue damage by overcoming the
10 toxin's inhibitory effects. The morphogens described
herein also are useful in therapies to treat damaged
neural pathways resulting from a neuropathy induced by
exposure to these toxins.

15

Example 6.   <u>Morphogen-Induced CAM Expression</u>

The morphogens described herein induce CAM
expression, particularly N-CAM expression, as part of
20 their induction of morphogenesis. CAMs are
morphoregulatory molecules identified in all tissues as
an essential step in tissue development. N-CAMs, which
comprise at least 3 isoforms (N-CAM-180, N-CAM-140 and
N-CAM-120, where "180", "140" and "120" indicate the
25 apparent molecular weights of the isoforms as measured
by polyacrylamide gel electrophoresis) are expressed at
least transiently in developing tissues, and
permanently in nerve tissue. Both the N-CAM-180 and N-
CAM-140 isoforms are expressed in both developing and
30 adult tissue. The N-CAM-120 isoform is found only in
adult tissue. Another neural CAM is L1.

N-CAMs are implicated in appropriate neural
development, including appropriate nuerulation,
35 neuronal migration, fasciculation, and synaptogenesis.

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Inhibition of N-CAM production, as by complexing the
molecule with an N-CAM-specific antibody, inhibits
retina organization, including retinal axon migration,
and axon regeneration in the peripheral nervous system,
5   as well as axon synapsis with target muscle cells. In
addition, significant evidence indicates that physical
or chemical trauma to neurons, oncogenic transformation
and some genetic neurological disorders are accompanied
by changes in CAM expression, which alter the adhesive
10   or migratory behavior of these cells. Specifically,
increased N-CAM levels are reported in Huntington's
disease striatum (e.g., striatal basal ganglia), and
decreased adhesion is noted in Alzheimer's disease.

15   The morphogens described herein can stimulate CAM
production, particularly L1 and N-CAM production,
including all three isoforms of the N-CAM molecule.
For example, N-CAM expression is stimulated
significantly in morphogen-treated NG108-15 cells.
20   Untreated NG108-15 cells exhibit a fibroblastic, or
minimally differentiated morphology and express only
the 180 and 140 isoforms of N-CAM normally associated
with a developing cell. Following morphogen treatment
these cells exhibit a morphology characteristic of
25   adult neurons and express enhanced levels of all three
N-CAM isoforms. Using a similar protocol as described
in the example below, morphogen treatment of NG108-15
cells also induced L1 expression.

30   In this example NG108-15 cells were cultured for
4 days in the presence of increasing concentrations of
OP-1 and standard Western blots performed on whole
cells extracts. N-CAM isoforms were detected with an
antibody which crossreacts with all three isoforms,
35   mAb H28.123, obtained from Sigma Chemical Co.,

St. Louis, the different isoforms being distinguishable
by their different mobilities on an electrophoresis
gel. Control NG108-15 cells (untreated) express both
the 140 kDa and the 180 kDa isoforms, but not the 120

5   kDa, as determined by western blot analyses using up to
100 μg of protein. Treatment of NG108-15 cells with
OP-1 resulted in a dose-dependent increase in the
expression of the 180 kDa and 140 kDa isoforms, as well
as the induction of the 120 kDa isoform. See Fig. 2A

10 and 2B. Fig. 2B is a Western blot of OP1-treated
NG108-15 cell extracts, probed with mAb H28.123,
showing the induction of all three isoforms. Fig. 2A
is a dose response curve of N-CAM-180 and N-CAM-140
induction as a function of morphogen concentration. N-

15 CAM-120 is not shown in the graph as it could not be
quantitated in control cells. However, as is clearly
evident from the Western blot in Fig. 2A, N-CAM-120 is
induced in response to morphogen treatment. The
differential induction of N-CAM 180 and 140 isoforms

20 seen may be because constitutive expression of the 140
isoform is close to maximum.

The increase in N-CAM expression corresponded in a
dose-dependent manner with the morphogen induction of

25 multicellular aggregates. Compare Fig. 2A and Fig 3.
Fig. 3 graphs the mean number of multilayered
aggregates (clumps) counted per 20 randomly selected
fields in 6 independent experiments, versus the
concentration of morphogen. The induction of the 120

30 isoform also indicates that morphogen-induced
redifferentiation of transformed cells stimulates not
only redifferentiation of these cells from a
transformed phenotype, but also differentiation to a
phenotype corresponding to a developed cell. Standard

35 immunolocalization studies performed with the mAb

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H28.123 on morphogen-treated cells show N-CAM cluster
formation associated with the periphery and processes
of treated cells and no reactivity with untreated
cells. Moreover, morphogen treatment does not appear
5   to inhibit cell division as determined by cell counting
or ^{3}H-thymidine uptake. Finally, known chemical
differentiating agents, such as Forskolin and
dimethylsulfoxide do not induce N-CAM production.

10   In addition, the cell aggregation effects of OP-1
on NG108-15 cells can be inhibited with anti-N-CAM
antibodies or antisense N-CAM oligonucleotides.
Antisense oligonucleotides can be made synthetically on
a nucleotide synthesizer, using standard means known in
15   the art. Preferably, phosphorothioate oligonucleotides
("S-oligos") are prepared, to enhance transport of the
nucleotides across cell membranes. Concentrations of
both N-CAM antibodies and N-CAM antisense
oliognucleotides sufficient to inhibit N-CAM induction
20   also inhibited formation of multilayered cell
aggregates. Specifically, incubation of morphogen-
treated NG108-115 cells with 0.3-3 μM N-CAM antisense
S-oligos, 5-500 μM unmodified N-CAM antisense oligos,
or 10 μg/ml mAb H28.123 significantly inhibits cell
25   aggregation. It is likely that morphogen treatment
also stimulates other CAMs, as inhibition is not
complete.

        The experiments also have been performed with
30   soluble morphogen (e.g., mature OP-1 associated with
its pro domain) which also specifically induced CAM
expression.

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The morphogens described herein are useful as therapeutic agents to treat neurological disorders associated with altered CAM levels, particularly N-CAM levels, such as Huntington's chorea and Alzheimers'
5   disease, and the like. In clinical applications, the morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

10   The efficacy of the morphogens described herein to affect N-CAM expression may be assessed _in_ _vitro_ using a suitable cell line and the methods described herein. In addition to a transformed cell line, N-CAM expression can be assayed in a primary cell culture of
15   neural or glial cells, following the procedures described herein. The efficacy of morphogen treatment on N-CAM expression _in_ _vivo_ may be evaluated by tissue biopsy as described in Example 9, below, and detecting N-CAM molecules with an N-CAM-specific antibody, such
20   as mAb H28.123, or using the animal model described in Example 11.

Alternatively, the level of N-CAM proteins or protein fragments present in cerebrospinal fluid or
25   serum also may be detected to evaluate the effect of morphogen treatment. N-CAM molecules are known to slough off cell surfaces and have been detected in both serum and cerebrospinal fluid. In addition, altered levels of the soluble form of N-CAM are associated with
30   normal pressure hydrocephalus and type II schizophrenia. N-CAM fluid levels may be detected following the procedure described in Example 9 and using an N-CAM specific antibody, such as mAb H28.123.

35

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Example 7. <u>Morphogen-Induced Nerve Gap Repair (PNS)</u>

The morphogens described herein also stimulate
peripheral nervous system axonal growth over extended
5 distances allowing repair and regeneration of damaged
neural pathways. While neurons of the peripheral
nervous system can sprout new processes following
injury, without guidance these sproutings typically
fail to connect appropriately and die. Where the break
10 is extensive, e.g., greater than 5 or 10 mm,
regeneration is poor or nonexistent.

In this example morphogen stimulation of nerve
regeneration was assessed using the rat sciatic nerve
15 model. The rat sciatic nerve can regenerate
spontaneously across a 5 mm gap, and occasionally
across a 10 mm gap, provided that the severed ends are
inserted in a saline-filled nerve guidance channel. In
this experiment, nerve regeneration across a 12mm gap
20 was tested.

Adult female Sprague-Dawley rats (Charles River
Laboratories, Inc.) weighing 230-250 g were
anesthetized with intraperitoneal injections of sodium
25 pentobarbital 35 mg/kg body weight). A skin incision
was made parallel and just posterior to the femur. The
avascular intermuscular plane between vastus lateralis
and hamstring muscles were entered and followed to the
loose fibroareolar tissue surrounding the sciatic
30 nerve. The loose tissue was divided longitudinally
thereby freeing the sciatic nerve over its full extent
without devascularizing any portion. Under a surgical

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microscope the sciatic nerves were transected with microscissors at mid-thigh and grafted with an OP-1 gel graft that separated the nerve stumps by 12 mm. The graft region was encased in a silicone tube 20 mm in

5 length with a 1.5 mm inner diameter,the interior of which was filled a morphogen solution. Specifically, The central 12 mm of the tube consisted of an OP-1 gel prepared by mixing 1 to 5 μg of substantially pure CHO-produced recombinant OP-1 with approximately 100 μl of

10 MATRIGEL^{TM} (from Collaborative Research, Inc., Bedford, MA), an extracellular matrix extract derived from mouse sarcoma tissue, and containing solubilized tissue basement membrane, including laminin, type IV collagen, heparin sulfate, proteoglycan and entactin, in

15 phosphate-buffered saline. The OP-1-filled tube was implanted directly into the defect site, allowing 4 mm on each end to insert the nerve stumps. Each stump was abutted against the OP-1 gel and was secured in the silicone tube by three stitches of commercially

20 available surgical 10-0 nylon through the epineurium, the fascicle protective sheath.

In addition to OP-1 gel grafts, empty silicone tubes, silicone tubes filled with gel only and

25 "reverse" autografts, wherein 12 mm transected segments of the animal's sciatic nerve were rotated 180° prior to suturing, were grafted as controls. All experiments were performed in quadruplicate. All wounds were closed by wound clips that were removed after 10 days.

30 All rats were grafted on both legs. At 3 weeks the animals were sacrificed, and the grafted segments removed and frozen on dry ice immediately. Frozen

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sections then were cut throughout the graft site, and
examined for axonal regeneration by immunofluorescent
staining using anti-neurofilament antibodies labeled
with flurocein (obtained from Sigma Chemical Co.,
5   St. Louis).

Regeneration of the sciatic nerve occurred across
the entire 12 mm distance in all graft sites wherein
the gap was filled with the OP-1 gel. By contrast,
10 empty silicone tubes and reverse autografts did not
show nerve regeneration, and only one graft site
containing the gel alone showed axon regeneration.

15 Example 8.   Morphogen-Induced Nerve Gap Repair (CNS)

Following axonal damage in vivo the CNS neurons are
unable to resprout processes. Accordingly, trauma to
CNS nerve tissue, including the spinal cord, optic
20 nerve and retina, severely damages or destroys the
neural pathways defined by these cells. Peripheral
nerve grafts have been used in an effort to bypass CNS
axonal damage. Successful autologous graft repair to
date apparently requires that the graft site occur near
25 the CNS neuronal cell body, and a primary result of CNS
axotomy is neuronal cell death. The efficacy of
morphogens described herein on CNS nerve repair, may be
evaluated using a rat crushed optic nerve model such as
the one described by Bignami et al., (1979) Exp. Eye
30 Res. 28: 63-69, the disclosure of which is incorporated
herein by reference. Briefly, and as described
therein, laboratory rats (e.g., from Charles River
Laboratories, Wilmington, MA) are anesthetized using
standard surgical procedures, and the optic nerve
35 crushed by pulling the eye gently out of the orbit,

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inserting a watchmaker forceps behind the eyeball and
squeezing the optic nerve with the forceps for
15 seconds, followed by a 30 second interval and second
15 second squeeze. Rats are sacrificed at different
5   time intervals, e.g., at 48 hours, and at 3, 4, 11, 15
and 18 days after operation. The effect of morphogen
on optic nerve repair can be assessed by performing the
experiment in duplicate and providing morphogen or PBS
(e.g., 25 µl solution, and 25 µg morphogen) to the
10   optic nerve, e.g., just prior to the operation,
concommitant with the operation, or at specific times
after the operation.

In the absence of therapy, the surgery induces
15   glial scarring of the crushed nerve, as determined by
immunofluoresence staining for glial fibrillary acidic
protein (GFA), a marker protein for glial scarring, and
by histology. Indirect immunofluoresence on air-dried
cryostat sections as described in Bignami et al. (1974)
20   J. Comp. Neur. 153: 27-38, using commercially
available antibodies to GFA (e.g., Sigma Chemical Co.,
St. Louis). Reduced levels of GFA are anticipated in
animals treated with the morphogen, evidencing the
ability of morphogens to inhibit glial scar formation
25   and to stimulate optic nerve regeneration.

Example 9.   Nerve Tissue Diagnostics

Morphogen localization in nerve tissue can be used
30   as part of a method for diagnosing a neurological
disorder or neuropathy. The method may be particularly
advantageous for diagnosing neuropathies of brain
tissue. Specifically, a biopsy of brain tissue is
performed on a patient at risk, using standard
35   procedures known in the medical art. Morphogen

expression associated with the biopsied tissue then is
assessed using standard methodologies, as by
immunolocalization, using standard immunofluorescence
techniques in concert with morphogen-specific antisera

5   or monoclonal antibodies. Specifically, the biopsied
tissue is thin sectioned using standard methodologies
known in the art, and fluorescently labelled (or
otherwise detectable) antibodies incubated with the
tissue under conditions sufficient to allow specific

10 antigen-antibody complex formation. The presence and
quantity of complex formed then is detected and
compared with a predetermined standard or reference
value. Detection of altered levels of morphogen
present in the tissue then may be used as an indicator

15 of tissue dysfunction. Alternatively, fluctuation in
morphogen levels may be assessed by monitoring
morphogen transcription levels, either by standard
northern blot analysis or in situ hybridization, using
a labelled probe capable of hybridizing specifically to

20 morphogen RNA and standard RNA hybridization protocols
well described in the art.

    Fluctuations in morphogen levels present in the
cerebrospinal fluid or bloodstream also may be used to

25 evaluate nerve tissue viability. For example,
morphogens are detected associated with adendema cells
which are known to secrete factors into the
cerebrospinal fluid, and are localized generally
associated with glial cells, and in the extracellular

30 matrix, but not with neuronal cell bodies.

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Accordingly, the cerebrospinal fluid may be a natural
means of morphogen transport. Alternatively,
morphogens may be released from dying cells into
cerebrospinal fluid. In addition, OP-1 recently has
5 been identified in human blood, which also may be a
means of morphogen transport, and/or a repository for
the contents of dying cells.

Spinal fluid may be obtained from an individual by
10 a standard lumbar puncture, using standard
methodologies known in the medical art. Similarly,
serum samples may be obtained by standard venipuncture
and serum prepared by centrifugation at 3,000 RPM for
ten minutes. The presence of morphogen in the serum or
15 cerebral spinal fluid then may be assessed by standard
Western blot (immunoblot), ELISA or RIA procedures.
Briefly, for example, with the ELISA, samples may be
diluted in an appropriate buffer, such as phosphate-
buffered saline, and 50 μl aliquots allowed to absorb
20 to flat bottomed wells in microtitre plates pre-coated
with morphogen-specific antibody, and allowed to
incubate for 18 hours at 4°C. Plates then may be
washed with a standard buffer and incubated with 50 μl
aliquots of a second morphogen-specific antibody
25 conjugated with a detecting agent, e.g., biotin, in an
appropriate buffer, for 90 minutes at room temperature.
Morphogen-antibody complexes then may be detected using
standard procedures.

30   Alternatively, a morphogen-specific affinity column
may be created using, for example, morphogen-specific
antibodies adsorbed to a column matrix, and passing the
fluid sample through the matrix to selectively extract
the morphogen of interest. The morphogen then is
35 eluted. A suitable elution buffer may be determined

empirically by determining appropriate binding and
elution conditions first with a control (e.g.,
purified, recombinantly-produced morphogen.) Fractions
then are tested for the presence of the morphogen by
5 standard immunoblot, and confirmed by N-terminal
sequencing. Morphogen concentrations in serum or other
fluid samples then may be determined using standard
portein quantification techniques, including by
spectrophotometric absorbance or by quantitation by
10 ELISA or RIA antibody assays. Using this procedure,
OP-1 has been identified in serum.

OP-1 was detected in human serum using the
following assay. A monoclonal antibody raised against
15 mammalian, recombinantly produced OP-1 using standard
immunology techniques well described in the art and
described generally in Example 13, was immobilized by
passing the antibody over an activated agarose gel
(e.g., Affi-Gel^{TM}, from Bio-Rad Laboratories, Richmond,
20 CA, prepared following manufacturer's instructions),
and used to purify OP-1 from serum. Human serum then
was passed over the column and eluted with 3M
K-thiocyanate. K-thiocyanante fractions then were
dialyzed in 6M urea, 20mM PO$_4$, pH 7.0, applied to a C8
25 HPLC column, and eluted with a 20 minute, 25-50%
acetonitrile/0.1% TFA gradient. Mature, recombinantly
produced OP-1 homodimers elute between 20-22 minutes.
Fractions then were collected and tested for the
presence of OP-1 by standard immunoblot. Fig. 4 is an
30 immunoblot showing OP-1 in human sera under reducing
and oxidized conditions. In the figure, lanes 1 and 4
are OP-1 standards, run under oxidized (lane 1) and

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reduced (lane 4) conditions. Lane 5 shows molecular
weight markers at 17, 27 and 39 kDa. Lanes 2 and 3 are
human sera OP-1, run under oxidized (lane 2) and
reduced (lane 3) conditions.

5

Morphogens may be used in diagnostic applications
by comparing the quantity of morphogen present in a
body fluid sample with a predetermined reference value,
with fluctuations in fluid morphogen levels indicating
10   a change in the status of nerve tissue. Alternatively,
fluctuations in the level of endogenous morphogen
antibodies may be detected by this method, most likely
in serum, using an antibody or other binding protein
capable of interacting specifically with the endogenous
15   morphogen antibody. Detected fluctuations in the
levels of the endogenous antibody may be used as
indicators of a change in tissue status.

20   Example 10.   <u>Alleviation of Immune Response-Mediated</u>
                    <u>Nerve Tissue Damage</u>

The morphogens described herein may be used to
alleviate immunologically-related damage to nerve
25   tissue. Details of this damage and the use of
morphogens to alleviate this injury are disclosed in
international application US92/07358 (WO93/04692). A
primary source of such damage to nerve tissue follows
hypoxia or ischemia-reperfusion of a blood supply to a
30   neural pathway, such as may result from an embolic
stroke, or may be induced during a surgical procedure.

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As described in international application US92/07358
(WO93/04692), morphogens have been shown to alleviate
damage to myocardial tissue following ischemia-
reperfusion of the blood supply to the tissue. The
5 effect of morphogens on alleviating immunologically-
related damage to nerve tissue may be assessed using
methodologies and models known to those skilled in the
art and described below.

10   For example, the rabbit embolic stroke model
provides a useful method for assessing the effect of
morphogens on tissue injury following cerebral
ischemia-reperfusion. The protocol disclosed below is
essentially that of Phillips et al. (1989) Annals of
15 Neurology 25:281-285, the disclosure of which is herein
incorporated by reference. Briefly, white New England
rabbits (2-3kg) are anesthetized and placed on a
respirator. The intracranial circulation then is
selectively catheterized by the Seldinger technique.
20 Baseline cerebral angiography then is performed,
employing a digital substration unit. The distal
internal carotid artery or its branches then is
selectively embolized with 0.035 ml of 18-hour-aged
autologous thrombus. Arterial occlusion is documented
25 by repeat angiography immediately after embolization.
After a time sufficient to induce cerebral infarcts (15
minutes or 90 minutes), reperfusion is induced by
administering a bolus of a reperfusion agent such as
the TPA analogue FB-FB-CF (e.g., 0.8 mg/kg over 2
30 minutes).

The effect of morphogen on cerebral infarcts can be
assessed by administering varying concentrations of
morphogens, e.g., OP-1, at different times following
35 embolization and/or reperfusion. The rabbits are

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sacrificed 3-14 days post embolization and their brains
prepared for neuropathological examination by fixing by
immersion in 10% neutral buffered formation for at
least 2 weeks. The brains then are sectioned in a
5   coronal plane at 2-3 mm intervals, numbered and
submitted for standard histological processing in
paraffin, and the degree of nerve tissue necrosis
determined visually. Morphogen-treated animals are
anticipated to reduce or significantly inhibit nerve
10   tissue necrosis following cerebral ischemia-reperfusion
in the test animals as determined by histology
comparison with nontreated animals.

Example 11.   Animal Model for Assessing Morphogen
15   Efficacy In Vivo

The in vivo activities of the morphogens described
herein also are assessed readily in an animal model as
described herein. A suitable animal, preferably
20   exhibiting nerve tissue damage, for example,
genetically or environmentally induced, is injected
intracerebrally with an effective amount of a morphogen
in a suitable therapeutic formulation, such as
phosphate-buffered saline, pH 7. The morphogen
25   preferably is injected within the area of the affected
neurons. The affected tissue is excised at a
subsequent time point and the tissue evaluated
morphologically and/or by evaluation of an appropriate
biochemical marker (e.g., by morphogen or N-CAM
30   localization; or by measuring the dose-dependent effect
on a biochemical marker for CNS neurotrophic activity
or for CNS tissue damage, using for example, glial
fibrillary acidic protein as the marker. The dosage

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and incubation time will vary with the animal to be
tested. Suitable dosage ranges for different species
may be determined by comparison with established animal
models.   Presented below is an exemplary protocol for
5   a rat brain stab model.

    Briefly, male Long Evans rats, obtained from
standard commercial sources, are anesthesized and the
head area prepared for surgery. The calvariae is
10 exposed using standard surgical procedures and a hole
drilled toward the center of each lobe using a 0.035K
wire, just piercing the calvariae. 25µl solutions
containing either morphogen (e.g., OP-1, 25µg) or PBS
then is provided to each of the holes by Hamilton
15 syringe. Solutions are delivered to a depth
approximately 3 mm below the surface, into the
underlying cortex, corpus callosum and hippocampus.
The skin then is sutured and the animal allowed to
recover.
20

    Three days post surgery, rats are sacrificed by
decapitation and their brains processed for sectioning.
Scar tissue formation is evaluated by immunofluoresence
staining for glial fibrillary acidic protein, a marker
25 protein for glial scarring, to qualitatively determine
the degree of scar formation. Glial fibrillary acidic
protein antibodies are available commercially, e.g.,
from Sigma Chemical Co., St. Louis, MO. Sections also
are probed with anti-OP-1 antibodies to determine the
30 presence of OP-1. Reduced levels of glial fibrillary
acidic protein are anticipated in the tissue sections
of animals treated with the morphogen, evidencing the
ability of morphogens to inhibit glial scar formation
and stimulate nerve regeneration.
35

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Example 12.   In Vitro Model for Evaluating Morphogen
               Species Transport Across the Blood-Brain
               Barrier.

5   Described below is an in vitro method for
evaluating the facility with which selected morphogen
species likely will pass across the blood-brain
barrier. A detailed description of the model and
protocol are provided by Audus et al. (1987) Ann. N.Y.
10 Acad. Sci. 507:9-18, the disclosure of which is
incorporated herein by reference.

        Briefly, microvessel endothelial cells are isolated
from the cerebral gray matter of fresh bovine brains.
15 Brains are obtained from a local slaughter house and
transported to the laboratory in ice cold minimum
essential medium (MEM) with antibiotics. Under sterile
conditions the large surface blood vessels and meninges
are removed using standard dissection procedures. The
20 cortical gray matter is removed by aspiration, then
minced into cubes of about 1mm. The minced gray matter
then is incubated with 0.5% dispase (BMB, Indianapolis,
IN) for 3 hours at 37° C in a shaking water bath.
Following the 3 hour digestion, the mixture is
25 concentrated by centrifugation (1000 x g for 10 min.),
then resuspended in 13% dextran and centrifuged for
10 min. at 5800 x g. Supernatant fat, cell debris and
myelin are discarded and the crude microvessel pellet
resuspended in 1 mg/ml collagenase/dispase and
30 incubated in a shaking water bath for 5 hours at 37° C.
After the 5-hour digestion, the microvessel suspension
is applied to a pre-established 50% Percoll gradient
and centrifuged for 10 min at 1000 x g. The band
containing purified endothelial cells (second band from
35 the top of the gradient) is removed and washed two

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times with culture medium (e.g., 50% MEM/50% F-12
nutrient mix). The cells are frozen (-80° C.) in
medium containing 20% DMSO and 10% horse serum for
later use.

5

After isolation, approximately 5×10^5 cells/cm^2
are plated on culture dishes or 5-12 mμ pore size
polycarbonate filters that are coated with rat collagen
and fibronectin. 10-12 days after seeding the cells,
10 cell monolayers are inspected for confluency by
microscopy.

Characterization of the morphological,
histochemical and biochemical properties of these cells
15 has shown that these cells possess many of the salient
features of the blood-brain barrier. These features
include: tight intercellular junctions, lack of
membrane fenestrations, low levels of pinocytotic
activity, and the presence of gamma-glutamyl
20 transpeptidase, alkaline phosphatase, and Factor VIII
antigen activities.

The cultured cells can be used in a wide variety of
experiments where a model for polarized binding or
25 transport is required. By plating the cells in
multi-well plates, receptor and non-receptor binding of
both large and small molecules can be conducted. In
order to conduct transendothelial cell flux
measurements, the cells are grown on porous
30 polycarbonate membrane filters (e.g., from Nucleopore,
Pleasanton, CA). Large pore size filters (5-12 mμ) are

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used to avoid the possibility of the filter becoming
the rate-limiting barrier to molecular flux. The use
of these large-pore filters does not permit cell growth
under the filter and allows visual inspection of the
5   cell monolayer.

Once the cells reach confluency, they are placed in
a side-by-side diffusion cell apparatus (e.g., from
Crown Glass, Sommerville, NJ). For flux measurements,
10 the donor chamber of the diffusion cell is pulsed with
a test substance, then at various times following the
pulse, an aliquot is removed from the receiver chamber
for analysis. Radioactive or fluorescently-labelled
substances permit reliable quantitation of molecular
15 flux. Monolayer integrity is simultaneously measured
by the addition of a non-transportable test substance
such as sucrose or inulin and replicates of at least 4
determinations are measured in order to ensure
statistical significance.
20

Example 13.   Screening Assay for Candidate Compounds
which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to
25 affect the level of a given morphogen may be found
using the following screening assay, in which the level
of morphogen production by a cell type which produces
measurable levels of the morphogen is determined with
and without incubating the cell in culture with the
30 compound, in order to assess the effects of the
compound on the cell. This can be accomplished by
detection of the morphogen either at the protein or RNA
level. A more detailed description also may be found
in international application US92/07359 (WO92/05172).
35

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13.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described
5 widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from
10 kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be
15 cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or
20 other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1
25 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor de
30 novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an ^{35}S-methionine/^{35}S-cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.
35

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13.2 Determination of Level of Morphogenic Protein

    In order to quantitate the production of a
morphogenic protein by a cell type, an immunoassay may
5 be performed to detect the morphogen using a polyclonal
or monoclonal antibody specific for that protein. For
example, OP-1 may be detected using a polyclonal
antibody specific for OP-1 in an ELISA, as follows.

10   1 μg/100 μl of affinity-purified polyclonal rabbit
IgG specific for OP-1 is added to each well of a
96-well plate and incubated at 37°C for an hour. The
wells are washed four times with 0.167M sodium borate
buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1%
15 Tween 20. To minimize non-specific binding, the wells
are blocked by filling completely with 1% bovine serum
albumin (BSA) in BSB and incubating for 1 hour at 37°C.
The wells are then washed four times with BSB
containing 0.1% Tween 20. A 100 μl aliquot of an
20 appropriate dilution of each of the test samples of
cell culture supernatant is added to each well in
triplicate and incubated at 37°C for 30 min. After
incubation, 100 μl biotinylated rabbit anti-OP-1 serum
(stock solution is about 1 mg/ml and diluted 1:400 in
25 BSB containing 1% BSA before use) is added to each well
and incubated at 37°C for 30 min. The wells are then
washed four times with BSB containing 0.1% Tween 20.
100 μl strepavidin-alkaline (Southern Biotechnology
Associates, Inc. Birmingham, Alabama, diluted 1:2000 in
30 BSB containing 0.1% Tween 20 before use) is added to
each well and incubated at 37°C for 30 min. The plates
are washed four times with 0.5M Tris buffered Saline

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(TBS), pH 7.2. 50μl substrate (ELISA Amplification
System Kit, Life Technologies, Inc., Bethesda, MD) is
added to each well incubated at room temperature for 15
min. Then, 50 μl amplifier (from the same

5   amplification system kit) is added and incubated for
another 15 min at room temperature. The reaction is
stopped by the addition of 50 μl 0.3 M sulphuric acid.
The OD at 490 nm of the solution in each well is
recorded. To quantitate OP-1 in culture media, a OP-1

10   standard curve is performed in parallel with the test
samples.

     Polyclonal antibody may be prepared as follows.
Each rabbit is given a primary immunization of 100

15   ug/500 μl E. coli produced OP-1 monomer (amino acids
328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500 μl
 Complete Freund's Adjuvant. The antigen is injected
subcutaneously at multiple sites on the back and flanks
of the animal. The rabbit is boosted after a month in

20   the same manner using incomplete Freund's Adjuvant.
Test bleeds are taken from the ear vein seven days
later. Two additional boosts and test bleeds are
performed at monthly intervals until antibody against
OP-1 is detected in the serum using an ELISA assay.

25   Then, the rabbit is boosted monthly with 100 μg of
antigen and bled (15 ml per bleed) at days seven and
ten after boosting.

     Monoclonal antibody specific for a given morphogen

30   may be prepared as follows. A mouse is given two
injections of E. coli produced OP-1 monomer. The first
injection contains 100μg of OP-1 in complete Freund's
adjuvant and is given subcutaneously. The second
injection contains 50 μg of OP-1 in incomplete adjuvant

35   and is given intraperitoneally. The mouse then

receives a total of 230 μg of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted

5 intraperitoneally with 100 μg of OP-1 (307-431) and 30 μg of the N-terminal peptide (Ser$_{293}$-Asn$_{309}$-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days

10 (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as

15 antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific
20 forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather
25 than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT:
        (A) NAME: CREATIVE BIOMOLECULES, INC.
        (B) STREET: 35 SOUTH STREET
        (C) CITY: HOPKINTON
        (D) STATE: MASSACHUSETTS
        (E) COUNTRY: USA
        (F) POSTAL CODE (ZIP): 01748
        (G) TELEPHONE: 1-508-435-9001
        (H) TELEFAX: 1-508-435-0454
        (I) TELEX:

    (ii) TITLE OF INVENTION: MORPHOGEN-INDUCED NERVE REGENERATION AND
        REPAIR

    (iii) NUMBER OF SEQUENCES: 33

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: CREATIVE BIOMOLECULES, INC.
        (B) STREET: 35 SOUTH STREET
        (C) CITY: HOPKINTON
        (D) STATE: MASSACHUSETTS
        (E) COUNTRY: USA
        (F) ZIP: 01748

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: KELLEY, ROBIN D.
        (B) REGISTRATION NUMBER: 34,637
        (C) REFERENCE/DOCKET NUMBER: CRP-070

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: 617/248-7000
        (B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 97 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(ix) FEATURE:
5   (A) NAME/KEY: Protein
            (B) LOCATION: 1..97
            (D) OTHER INFORMATION: /label= GENERIC-SEQ1
                    /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
                    ONE OF THE 20 NATURALLY-OCCURING L-ISOMER, A-AMINO
10   ACIDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15   Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        1   5   10   15

        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
                    20   25   30
20
        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                35   40   45

        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
25   50   55   60

        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        65   70   75   80

30   Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
                        85   90   95

        Xaa

35
    (2) INFORMATION FOR SEQ ID NO:2:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 97 amino acids
40   (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: protein
45

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(ix) FEATURE:
    (A) NAME/KEY: Protein
    (B) LOCATION: 1..97
    (D) OTHER INFORMATION: /label= GENERIC-SEQ2
5   /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
            ONE OF THE 20 NATURALLY OCCURING L-ISOMER A-AMINO
            ACIDS, OR A DERIVATIVE THEREOF."

10   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1   5   10   15

15   Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
           20   25   30

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      35   40   45
20
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
      50   55   60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
25   65   70   75   80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
           85   90   95

30   Xaa

(2) INFORMATION FOR SEQ ID NO:3:

35   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 97 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear
40
(ii) MOLECULE TYPE: protein

(ix) FEATURE:
45   (A) NAME/KEY: Protein
        (B) LOCATION: 1..97
        (D) OTHER INFORMATION: /label= GENERIC-SEQ3
            /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
            FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
50   AS DEFINED IN THE SPECIFICATION."

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Ala
1               5               10              15

Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
        20              25              30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Leu
        35              40              45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
    50              55              60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65              70              75              80

Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Gly Cys
            85              90              95

Xaa
```

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..102
        (D) OTHER INFORMATION: /label= GENERIC-SEQ4
            /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
            FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
            AS DEFINED IN THE SPECIFICATION."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
1               5               10              15

Xaa Trp Xaa Xaa Ala Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
        20              25              30
```

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```
        Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
                35              40              45
```

```
   5    Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                50              55              60
```

```
        Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
        65              70              75              80
```

```
  10    Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
                    85              90              95
```

```
        Xaa Xaa Cys Gly Cys Xaa
                100
```

15

(2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 139 amino acids
  20   (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein
  25
        (vi) ORIGINAL SOURCE:
            (A) ORGANISM: Homo sapiens
            (F) TISSUE TYPE: HIPPOCAMPUS

  30   (ix) FEATURE:
            (A) NAME/KEY: Protein
            (B) LOCATION: 1..139
            (D) OTHER INFORMATION: /label= hOP1-MATURE

  35
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
        Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
        1               5               10              15
  40
        Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser
                20              25              30
```

```
        Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
  45            35              40              45
```

```
        Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
                50              55              60
```

```
  50    Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
        65              70              75              80
```

```
            Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
                        85                  90              95

            Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
  5                     100             105             110

            Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
                        115             120             125

 10         Arg Asn Met Val Val Arg Ala Cys Gly Cys His
                    130             135
```

   (2) INFORMATION FOR SEQ ID NO:6:

 15   (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 139 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS: single
               (D) TOPOLOGY: linear
 20
          (ii) MOLECULE TYPE: protein

          (vi) ORIGINAL SOURCE:
               (A) ORGANISM: MURIDAE
 25   (F) TISSUE TYPE: EMBRYO

          (ix) FEATURE:
               (A) NAME/KEY: Protein
               (B) LOCATION: 1..139
 30   (D) OTHER INFORMATION: /label= MOP1-MATURE

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
 35         Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
            1               5                  10              15

            Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser
                        20                  25              30
 40
            Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
                        35                  40              45

            Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
 45                     50                  55              60

            Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
            65                  70                  75                  80

 50         Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
                        85                  90                  95
```

```
            Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
                        100             105                 110

            Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
    5               115                 120                 125

            Arg Asn Met Val Val Arg Ala Cys Gly Cys His
                130             135
```

10   (2) INFORMATION FOR SEQ ID NO:7:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 139 amino acids
        (B) TYPE: amino acid
15   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

20   (vi) ORIGINAL SOURCE:
        (A) ORGANISM: HOMO SAPIENS
        (F) TISSUE TYPE: HIPPOCAMPUS

     (ix) FEATURE:
25   (A) NAME/KEY: Protein
        (B) LOCATION: 1..139
        (D) OTHER INFORMATION: /label= HOP2-MATURE

30   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
            Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
            1               5               10                  15

            Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
                        20              25                  30

            His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
                        35              40                  45

            Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
                50              55                  60

            Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
            65              70                  75                  80

            Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
                        85                  90                  95

            Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
                        100             105                 110
```
35

40

45

50

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```
        Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
                115             120             125

        Arg Asn Met Val Val Lys Ala Cys Gly Cys His
            130             135
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 139 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
       (A) ORGANISM: MURIDAE
       (F) TISSUE TYPE: EMBRYO

    (ix) FEATURE:
       (A) NAME/KEY: Protein
       (B) LOCATION: 1..139
       (D) OTHER INFORMATION: /label= MOP2-MATURE

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
        Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
        1               5               10              15

        Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
                    20              25              30

        Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
                35              40              45

        Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
            50              55              60

        Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
        65              70              75              80

        Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
                        85              90              95
```

```
        Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
                    100             105                 110

        Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
  5             115             120                 125

        Arg Asn Met Val Val Lys Ala Cys Gly Cys His
            130                 135
```

10 (2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 101 amino acids
            (B) TYPE: amino acid
  15   (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

20   (vi) ORIGINAL SOURCE:
            (A) ORGANISM: bovinae

        (ix) FEATURE:
            (A) NAME/KEY: Protein
  25   (B) LOCATION: 1..101
            (D) OTHER INFORMATION: /label= CBMP-2A-FX

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
30
```
        Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
        1               5               10              15

        Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly
  35                20              25              30

        Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
                35              40              45

40      Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala
            50              55              60

        Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
        65              70              75              80
  45
```

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```
         Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu
                         85                  90              95
```

```
5        Gly Cys Gly Cys Arg
                         100
```

(2) INFORMATION FOR SEQ ID NO:10:

10
    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 101 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

15
    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
      (A) ORGANISM: HOMO SAPIENS
      (F) TISSUE TYPE: hippocampus
20
    (ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..101
      (D) OTHER INFORMATION: /label= CBMP-2B-FX
25

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
         Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
30       1               5               10              15
```

```
         Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
                         20                  25              30
```

```
35       Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
                     35                  40              45
```

```
         Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
                 50                  55              60
40
```

```
         Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
         65                  70                  75              80
```

```
         Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
45                       85                  90              95
```

```
         Gly Cys Gly Cys Arg
                         100
```

        (2) INFORMATION FOR SEQ ID NO:11:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 102 amino acids
   5   (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

           (ii) MOLECULE TYPE: protein
   10
           (vi) ORIGINAL SOURCE:
                (A) ORGANISM: DROSOPHILA MELANOGASTER

           (ix) FEATURE:
   15   (A) NAME/KEY: Protein
                (B) LOCATION: 1..101
                (D) OTHER INFORMATION: /label= DPP-FX

   20   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

            Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
            1   5   10   15

   25   Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
                        20   25   30

            Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
                    35   40   45
   30
            Val Val Gln Thr Leu Val Asn Asn Asn Pro Gly Lys Val Pro Lys
                    50   55   60

            Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
   35   65   70   75   80

            Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
                            85   90   95

   40   Val Gly Cys Gly Cys Arg
                        100

        (2) INFORMATION FOR SEQ ID NO:12:

   45   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 102 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
   50

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(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
        (A) ORGANISM: XENOPUS

5

(ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..102
        (D) OTHER INFORMATION: /label= VGL-FX

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln
1               5                   10                  15

Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly
            20                  25                  30

Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala
        35                  40                  45

Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu
        50                  55                  60

Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr
65                  70                  75                  80

Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val
                85                  90                  95

Asp Glu Cys Gly Cys Arg
                100
```

15

20

25

30

35 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
        (B) TYPE: amino acid
40   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (vi) ORIGINAL SOURCE:
        (A) ORGANISM: MURIDAE

(ix) FEATURE:
        (A) NAME/KEY: Protein
50   (B) LOCATION: 1..102
        (D) OTHER INFORMATION: /label= VGR-1-FX

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln
1               5                   10                  15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
            20              25                  30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
        35              40                  45

Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
        50              55                  60

Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
65              70                  75                  80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
                85                  90                  95

Arg Ala Cys Gly Cys His
                100
```

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 106 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens
        (F) TISSUE TYPE: brain

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..106
        (D) OTHER INFORMATION: /note= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His
1               5                   10                  15
```

```
        Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly
                    20              25              30

        Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala
  5             35              40              45

        Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly
                50           .  55              60

 10     Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser
        65              70              75                      80

        Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu
                        85              90              95
 15
        Asp Met Val Val Asp Glu Cys Gly Cys Arg
                    100             105
```

    (2) INFORMATION FOR SEQ ID NO:15:
 20
        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 5 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
 25   (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

 30
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

        Cys Xaa Xaa Xaa Xaa
        1   5
 35
    (2) INFORMATION FOR SEQ ID NO:16:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 1822 base pairs
 40   (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA
 45
        (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

 50   (vi) ORIGINAL SOURCE:
            (A) ORGANISM: HOMO SAPIENS
            (F) TISSUE TYPE: HIPPOCAMPUS

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10
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

        GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG   57
                                                           Met His Val
15   1

        CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA   105
        Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
                 5   10   15
20
        CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC   153
        Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
         20   25   30   35

25   GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG   201
        Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
                         40   45   50

        CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC   249
30   Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
                         55   60   65

        CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG   297
        Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
35   70   75   80

        CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC   345
        Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
                 85   90   95
40
        GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC   393
        Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
        100   105   110   115

45   CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC   441
        Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
                         120   125   130

        ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC   489
50   Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
                         135   140   145

```
    CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC      537
    His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile
            150                 155                 160

 5  CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC      585
    Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp
            165                 170                 175

    TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT      633
10  Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr
    180                 185                 190                 195

    CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC      681
    Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu
15                  200                 205                 210

    GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC      729
    Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp
                    215                 220                 225
20
    ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG      777
    Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu
                    230                 235                 240

25  GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC      825
    Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro
            245                 250                 255

    AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC      873
30  Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro
    260                 265                 270                 275

    TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC      921
    Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile
35                  280                 285                 290

    CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC      969
    Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro
                    295                 300                 305
40
    AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC     1017
    Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser
                    310                 315                 320

45  AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC     1065
    Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
            325                 330                 335

    CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC     1113
50  Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala
    340                 345                 350                 355
```

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```
    GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG        1161
    Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met
                360                     365                 370

 5  AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC        1209
    Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn
                375                     380                 385

    CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC        1257
10  Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
                390                     395                 400

    ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA        1305
    Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
15      405                     410                 415

    TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC             1351
    Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
    420                 425                 430
20
    GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG     1411

    GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG     1471

25  TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC     1531

    ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC     1591

    GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT     1651
30
    CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG     1711

    GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC     1771

35  CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A              1822
```

(2) INFORMATION FOR SEQ ID NO:17:

40   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 431 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

45   (ii) MOLECULE TYPE: protein

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

    Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
50 1   5   10   15

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```
      Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
               20              25                  30

      Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
  5          35              40              45

      Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
           50              55                  60

 10   Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
      65              70                  75                      80

      Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
                   85              90                      95
 15
      Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
                   100             105             110

      Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
 20       115             120                 125

      Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
          130             135             140

 25   Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
      145             150             155             160

      Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
                   165             170             175
 30
      Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
                   180             185             190

      Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
 35            195             200             205

      Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
          210             215             220

 40   Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
      225             230             235             240

      His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
                   245             250             255
 45
      Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
                   260             265             270

      Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
 50       275             280             285
```

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```
        Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
            290             295             300
 
        Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
 5      305             310             315             320

        Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
                        325             330             335

10      Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
                        340             345             350

        Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
                        355             360             365
15
        Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
            370             375             380

        Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
20      385             390             395             400

        Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
                        405             410             415

25      Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
                        420             425             430
```

(2) INFORMATION FOR SEQ ID NO:18:

30   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 1873 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear
35
            (ii) MOLECULE TYPE: cDNA

            (iii) HYPOTHETICAL: NO

40   (iv) ANTI-SENSE: NO

            (vi) ORIGINAL SOURCE:
                (A) ORGANISM: MURIDAE
                (F) TISSUE TYPE: EMBRYO
45
            (ix) FEATURE:
                (A) NAME/KEY: CDS
                (B) LOCATION: 104..1393
                (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
50   /product= "MOP1"
                        /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG     60

  5   CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC    115
                                                     Met His Val Arg
                                                      1

      TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT    163
 10   Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
       5               10                  15                  20

      CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG    211
 15   Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
                       25                  30                  35

      GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG    259
      Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
                       40                  45                  50
 20
      GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG    307
      Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro
                   55                  60                  65

 25   CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG    355
      Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu
                   70                  75                  80

      GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG    403
 30   Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln
       85                  90                  95                 100

      GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT    451
      Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro
 35                   105                 110                 115

      TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC    499
      Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val
                       120                 125                 130
 40
      ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT    547
      Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro
                       135                 140                 145

 45   CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG    595
      Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu
                   150                 155                 160

      GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC    643
 50   Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile
      165                 170                 175                 180
```

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```
    CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG          691
    Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val
                        185                 190                 195

 5  CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC          739
    Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser
                        200                 205                 210

    CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA          787
10  Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr
                        215                 220                 225

    GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA          835
    Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu
15          230                 235                 240

    CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG          883
    Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu
    245                 250                 255                 260
20
    GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG          931
    Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met
                        265                 270                 275

25  GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC          979
    Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser
                        280                 285                 290

    ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC          1027
30  Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn
                        295                 300                 305

    CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC          1075
    Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp
35      310                 315                 320

    CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC          1123
    Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp
    325                 330                 335                 340
40
    CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC          1171
    Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr
                        345                 350                 355

45  TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC          1219
    Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala
                        360                 365                 370

    ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC          1267
50  Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp
                        375                 380                 385
```

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```
      ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT    1315
      Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser
          390             395             400

  5   GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC GAC CTG AAG AAG TAC AGA    1363
      Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu Lys Lys Tyr Arg
      405             410             415             420

      AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG       1413
 10   Asn Met Val Val Arg Ala Cys Gly Cys His
                  425             430

      ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG   1473

 15   CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG   1533

      AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT   1593

      GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT   1653
 20
      GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGAGT   1713

      AATCGCAAGC CTCGTTCAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG   1773

 25   TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT   1833

      GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC                         1873
```

 30   (2) INFORMATION FOR SEQ ID NO:19:

           (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 430 amino acids
               (B) TYPE: amino acid
 35   (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: protein

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
 40
      Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
       1   5   10   15

      Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 45   20   25   30

      Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
                  35   40   45

 50   Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
                  50   55   60

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```
     Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
     65              70              75              80

  5  Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
                     85              90              95

     Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
                 100             105             110

 10  Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
                 115             120             125

     Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
                 130             135             140
 15
     Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
     145             150             155             160

     Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
 20              165             170             175

     Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
                 180             185             190

 25  Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
                 195             200             205

     Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
         210             215             220
 30
     Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
     225             230             235             240

     Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 35              245             250             255

     Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
                 260             265             270

 40  Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
                 275             280             285

     Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
         290             295             300
 45
     Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
     305             310             315             320

     Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 50              325             330             335
```

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```
        Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
                340             345             350
```

```
   5    Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
                355             360             365
```

```
        Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
                370             375             380
```

```
  10    Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
        385             390             395             400
```

```
        Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu
                405             410             415
```

```
  15    Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
                420             425             430
```

```
        (2) INFORMATION FOR SEQ ID NO:20:
  20
            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 1723 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
  25            (D) TOPOLOGY: linear

           (ii) MOLECULE TYPE: cDNA

           (vi) ORIGINAL SOURCE:
  30            (A) ORGANISM: Homo sapiens
                (F) TISSUE TYPE: HIPPOCAMPUS

           (ix) FEATURE:
                (A) NAME/KEY: CDS
  35            (B) LOCATION: 490..1696
                (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
                    /product= "hOP2-PP"
                    /note= "hOP2 (cDNA)"

  40
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

        GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA        60

  45    GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC       120

        CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC       180

        GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT       240
  50
```

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```
CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG      300
GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC      360
5  CGCCCCGCCC CGCCGCCCGC CGCCCGCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC    420
AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC      480
```

```
   CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG       528
10          Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
             1               5                   10
```

```
GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC       576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro
15       15                20                  25
```

```
GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG       624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln
30            35                  40                  45
20
CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC       672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg
               50                  55                  60
```

```
25  GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG     720
Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met
                 65                  70                  75
```

```
CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG      768
30 Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala
                 80                  85                  90
```

```
CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT     816
Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val
35     95                  100                 105
```

```
AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG      864
Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp
110                 115                 120                 125
40
AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC      912
Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val
                 130                 135                 140
```

```
45  ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC    960
Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu
                 145                 150                 155
```

```
AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC     1008
50 Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser
                 160                 165                 170
```

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```
    AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT         1056
    Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala
        175             180             185

  5 GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC         1104
    Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys
    190             195             200             205

    TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG         1152
 10 Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu
                210             215             220

    ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT         1200
    Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly
 15             225             230             235

    CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG         1248
    Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg
            240             245             250
 20
    GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG         1296
    Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg
        255             260             265

 25 AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC         1344
    Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu
    270             275             280             285

    CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC         1392
 30 Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys
                290             295             300

    CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC         1440
    Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp
 35             305             310             315

    TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG         1488
    Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu
                320             325             330
 40
    TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC         1536
    Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile
        335             340             345

 45 CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG         1584
    Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala
    350             355             360             365
```

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```
    TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC      1632
    Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp
                    370                 375             380

  5 AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC AAG      1680
    Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys
                    385                 390             395

    GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG               1723
 10 Ala Cys Gly Cys His
                    400
```


15 (2) INFORMATION FOR SEQ ID NO:21:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 402 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear
20

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
25 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
    1               5               10                  15

   Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
                20                  25                  30
30
   Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
            35                  40                  45

   Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
35      50                  55                  60

   Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
    65                  70                  75                  80

40 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
                    85                  90                  95

   Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
                100                 105                 110
45
   Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
            115                 120                 125

   Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
50      130                 135                 140
```

```
        Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
        145                 150             155                 160

  5     Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
                        165             170             175

        Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
                    180             185             190

  10    Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
                    195             200             205

        Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
            210             215             220
  15
        Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
        225             230             235             240

        Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
  20            245             250             255

        Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
                    260             265             270

  25    Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
                275             280             285

        Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
            290             295             300
  30
        Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
        305             310             315             320

        Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
  35                    325             330             335

        Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
                    340             345             350

  40    Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
                    355             360             365

        Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
            370             375             380
  45
        Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly
        385             390             395             400

        Cys His
  50
```

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(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1926 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: MURIDAE
        (F) TISSUE TYPE: EMBRYO

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 93..1289
        (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
            /product= "mOP2-PP"
            /note= "mOP2 cDNA"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT      60

ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA      113
                                   Met Ala Met Arg Pro Gly Pro
                                    1               5

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT      161
Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly
         10                  15                  20

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG      209
Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu
     25                  30                  35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA      257
Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly
 40                  45                  50                  55

CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC      305
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser
                 60                  65                  70

GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC      353
Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp
                 75                  80                  85

GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG      401
Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met
                 90                  95                  100
```

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```
    AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG    449
    Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu
        105             110             115

 5  CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG    497
    Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly
    120             125             130             135

    GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC ACC    545
10  Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr
                140             145             150

    CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC CAA    593
    His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Gln
15              155             160             165

    GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG    641
    Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr
                170             175             180
20
    CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA GCC    689
    Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala
        185             190             195

25  AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC CTC    737
    Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu
    200             205             210             215

    TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT GGT    785
30  Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly
                220             225             230

    CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA ACC    833
    Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr
35              235             240             245

    TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG AGA    881
    Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg
        250             255             260
40
    CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC CCC    929
    Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro
        265             270             275

45  AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA    977
    Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg
    280             285             290             295

    GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC   1025
50  Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly
                300             305             310
```

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```
    TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT     1073
    Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys
                315                 320                 325

 5  GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC     1121
    Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn
                330                 335                 340

    CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC     1169
 10 His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val
                345                 350                 355

    CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG     1217
    Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu
 15 360                 365                 370                 375

    TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG     1265
    Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met
                380                 385                 390
 20
    GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC TGCTTCTACT    1319
    Val Val Lys Ala Cys Gly Cys His
                395

 25 ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT   1379

    CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA AAATTCTGGT   1439

    CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC   1499
 30
    TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT   1559

    CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC   1619

 35 AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT   1679

    CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTTAGGT ATAACAGACA CATACACTTA   1739

    GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG   1799
 40
    CCAGGTATAG CGGTGCATGT CATTAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT   1859

    CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAC   1919

 45 GGAATTC                                                            1926
```

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(2) INFORMATION FOR SEQ ID NO:23:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 399 amino acids
5   (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

10   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
    Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
    1               5                   10                  15

15  Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
                20                  25                  30

    Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu
            35                  40                  45
20
    Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala
        50                  55                  60

    Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
25  65                  70                  75                  80

    His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu
                85                  90                  95

30  Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
                100                 105                 110

    Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
            115                 120                 125
35
    Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
        130                 135                 140

    Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
40  145                 150                 155                 160

    Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
                165                 170                 175

    Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu
45              180                 185                 190

    Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
        195                 200                 205
50
    Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser
        210                 215                 220
```

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```
     Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
     225             230             235             240

  5  Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
                     245             250             255

     Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
                 260             265             270

 10  Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
             275             280             285

     Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr
         290             295             300
 15
     Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
     305             310             315             320

     Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp
 20              325             330             335

     Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His
             340             345             350

 25  Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
             355             360             365

     Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile
         370             375             380
 30
     Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
     385             390             395
```

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1368 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..1368

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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```
    ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC        48
    Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
     1               5                  10                 15

  5 CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG        96
    Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
                    20                 25                 30

    GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC       144
 10 Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
                    35                 40                 45

    CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC       192
    Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
 15     50                 55                 60

    TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC       240
    Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
     65                 70                 75                 80
 20
    CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG       288
    Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
                    85                 90                 95

 25 CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG       336
    Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
                    100                105                110

    GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC       384
 30 Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
                    115                120                125

    GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC       432
    Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
 35     130                135                140

    CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG       480
    Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
    145                150                155                160
 40
    AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT       528
    Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
                    165                170                175

 45 CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG       576
    Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
                    180                185                190

    ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG       624
 50 Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
                    195                200                205
```

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```
    ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC        672
    Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
        210                 215                 220

 5  ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC        720
    Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
    225                 230                 235                 240

    GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC        768
10  Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
                    245                 250                 255

    GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA        816
    Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
15                  260                 265                 270

    CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA        864
    His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
                    275                 280                 285
20
    CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC        912
    Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
                290                 295                 300

25  TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC        960
    Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
    305                 310                 315                 320

    CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG       1008
30  His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
                    325                 330                 335

    GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC       1056
    Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
35                  340                 345                 350

    AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG       1104
    Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
                    355                 360                 365
40
    CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC       1152
    His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
                    370                 375                 380

45  GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT       1200
    Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
    385                 390                 395                 400

    GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC       1248
50  Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
                    405                 410                 415
```

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```
    AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC      1296
    Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
                420                 425                 430

 5  CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT      1344
    His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
                435                 440                 445

    GTG AAA TCC TGC GGG TGC CAT TGA                                      1368
10  Val Lys Ser Cys Gly Cys His
                450                 455
```

(2) INFORMATION FOR SEQ ID NO:25:
15

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 455 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear
20

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
25  Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
     1               5                10                15

    Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
                20                25                30
30
    Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
                35                40                45

    Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
35       50                55                60

    Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
    65                70                75                80

40  Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
                85                90                95

    Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
                100               105               110
45
    Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
                115               120               125

    Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
50       130               135               140
```

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```
    Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
    145             150             155             160

 5  Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
                165             170             175

    Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
                180             185             190

10  Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
                195             200             205

    Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
                210             215             220
15
    Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
    225             230             235             240

    Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
20              245             250             255

    Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
                260             265             270

25  His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
                275             280             285

    Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
                290             295             300
30
    Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
    305             310             315             320

    His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
35              325             330             335

    Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
                340             345             350

40  Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
                355             360             365

    His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
                370             375             380
45
    Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
    385             390             395             400

    Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
50              405             410             415
```

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```
     Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
                 420             425             430

     His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
5        435             440                 445

     Val Lys Ser Cys Gly Cys His
         450             455

10
     (2) INFORMATION FOR SEQ ID NO:26:

             (i) SEQUENCE CHARACTERISTICS:
                 (A) LENGTH: 104 amino acids
15               (B) TYPE: amino acid
                 (C) STRANDEDNESS: single
                 (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: protein
20

            (ix) FEATURE:
                 (A) NAME/KEY: Protein
                 (B) LOCATION: 1..104
25               (D) OTHER INFORMATION: /note= "BMP3"


            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

30       Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
         1               5               10                  15

         Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly
                         20              25                  30
35
         Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
                 35              40                  45

         Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Pro Gly Ile
40           50              55                  60

         Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
         65              70                  75                  80

45       Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
                         85                  90                  95

         Thr Val Glu Ser Cys Ala Cys Arg
                         100
50
```

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(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: HOMO SAPIENS

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..102
        (D) OTHER INFORMATION: /note= "BMP5"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1               5               10                  15

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
            20              25                  30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
        35              40              45

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
    50              55                  60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65              70                  75                  80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
            85                  90                  95

Arg Ser Cys Gly Cys His
            100
```

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

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(vi) ORIGINAL SOURCE:
        (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..102
        (D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1               5                   10                  15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
            20                  25                  30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
        35                  40                  45

Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
        50                  55                  60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65                  70                  75                  80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
                85                  90                  95

Arg Ala Cys Gly Cys His
                100
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..102
        (D) OTHER INFORMATION: /label= OPX
                /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
                FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
                AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1               5               10                  15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
            20              25              30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
        35          40              45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
    50              55              60

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65              70              75                  80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
        85              90                  95

Xaa Ala Cys Gly Cys His
        100
```

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 97 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..97
        (D) OTHER INFORMATION: /label= GENERIC-SEQ5
                /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
                FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
                AS DEFINED IN THE SPECIFICATION."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Xaa
1               5               10                  15

Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
            20              25              30
```

```
    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa
        35                      40              45

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
5       50                  55              60

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    65                  70              75                      80

10  Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
                        85              90                  95

    Xaa

15
```

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 102 amino acids
20   (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein
25

    (ix) FEATURE:
        (A) NAME/KEY: Protein
        (B) LOCATION: 1..102
30   (D) OTHER INFORMATION: /label= GENERIC-SEQ6
            /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
            FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
            AS DEFINED IN THE SPECIFICATION. "

35

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
    Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
    1               5               10                  15
40
    Xaa Trp Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
            20                  25              30

    Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
45      35                  40              45

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        50                  55              60

50  Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
    65                  70              75                      80
```

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```
     Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
                  85                 90                  95
```

```
     Xaa Xaa Cys Xaa Cys Xaa
  5              100
```

(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
10   (A) LENGTH: 1247 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

15   (ii) MOLECULE TYPE: cDNA

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: HOMO SAPIENS
        (F) TISSUE TYPE: BRAIN
20
    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 84..1199
        (D) OTHER INFORMATION: /product= "GDF-1"
25   /note= "GDF-1 CDNA"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
30 GGGGACACCG GCCCCGCCCT CAGCCCACTG GTCCCGGGCC GCCGCGGACC CTGCGCACTC       60

   TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC        110
                            Met Pro Pro Pro Gln Gln Gly Pro Cys
                             1               5
35
   GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC      158
   Gly His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
    10              15                  20              25

40 CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG      206
   Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln
                30                  35                  40

   GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG      254
45 Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro
                45                  50                  55

   GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG      302
   Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu
50              60                  65                  70
```

```
        ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC ACC CTG CAA CCG        350
        Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val Thr Leu Gln Pro
             75                  80                  85

     5  TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC        398
        Cys His Val Glu Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile
             90                  95                 100                 105

        CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG        446
    10  Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala
                            110                 115                 120

        GGG CAT TGC CCT GAG TGG ACA GTC GTC TTC GAC CTG TCG GCT GTG GAA        494
        Gly His Cys Pro Glu Trp Thr Val Val Phe Asp Leu Ser Ala Val Glu
    15                  125                 130                 135

        CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG        542
        Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala
                       140                 145                 150
    20
        GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA        590
        Ala Ala Ala Ala Ala Pro Glu Gly Gly Trp Glu Leu Ser Val Ala Gln
                       155                 160                 165

    25  GCG GGC CAG GGC GCG GGC GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG        638
        Ala Gly Gln Gly Ala Gly Ala Asp Pro Gly Pro Val Leu Leu Arg Gln
        170                 175                 180                 185

        TTG GTG CCC GCC CTG GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC        686
    30  Leu Val Pro Ala Leu Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala
                            190                 195                 200

        GCT TGG GCT CGC AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG        734
        Ala Trp Ala Arg Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu
    35                  205                 210                 215

        GCG CTA CGC CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC        782
        Ala Leu Arg Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala
                  220                 225                 230
    40
        TCG CTG CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC        830
        Ser Leu Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala
                  235                 240                 245

    45  CGG CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC        878
        Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly
        250                 255                 260                 265

        GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC GAG GTG GGC TGG        926
    50  Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp
                       270                 275                 280
```

```
     CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG        974
     His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln
                 285                     290                 295

  5  GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG       1022
     Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro
                 300                     305                 310

     GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG       1070
  10 Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro
                 315                     320                 325

     GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC       1118
     Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile
  15 330                     335                 340                 345

     TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT       1166
     Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr
                         350                 355                 360
  20
     GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGA     1219
     Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg
                 365                     370

  25 CCCGGGCCCA ACAATAAATG CCGCGTGG                                        1247
```

(2) INFORMATION FOR SEQ ID NO:33:

30   (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 372 amino acids
               (B) TYPE: amino acid
               (D) TOPOLOGY: linear

35   (ii) MOLECULE TYPE: protein

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
     Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu Leu
  40  1               5                   10                  15

     Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro
                 20                  25                  30

  45 Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu
                 35                  40                  45

     Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg
             50                  55                  60
  50
     Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg
         65                  70                  75                  80
```

```
        Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly
                        85                90                    95

     5  Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr
                    100               105               110

        Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr
                115               120               125

    10  Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg
                130               135               140

        Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Ala Pro Glu
        145               150               155               160
    15
        Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala
                        165               170               175

        Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro
    20                  180               185               190

        Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser
                195               200               205

    25  Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro
                210               215               220

        Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu
        225               230               235               240
    30
        Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu
                        245               250               255

        Pro Val Leu Gly Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu
    35              260               265               270

        Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
                275               280               285

    40  Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val
                290               295               300

        Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu
        305               310               315               320
    45
```

```
    Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
                    325             330             335

    Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
5                   340             345             350

    Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
                    355             360             365

10  Cys Gly Cys Arg
        370
```

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What is claimed is:

1. The use of a morphogen in the manufacture of a
   pharmaceutical for enhancing survival of neural
   5   cells at risk of dying.

2. A method for enhancing survival of neural cells at
   risk of dying, the method comprising providing a
   morphogen to said cells at a concentration and for
   10   a time sufficient to enhance survival of said
   cells.

3. The invention of claim 1 or 2 wherein said cells
   are at risk of dying due to chemical or mechanical
   15   trauma to nerve tissue comprising said cells.

4. The invention of claim 3 wherein said trauma
   comprises a transected nerve.

20 5. The invention of claim 3 wherein said morphogen is
   provided to said cells prior to said trauma.

6. The invention of claim 3 wherein said trauma
   results in demyelination of said cells.
25

7. The invention of claim 3 wherein said trauma
   results from exposure of said cells to a cellular
   toxin.

30 8. The invention of claim 7 wherein said toxin
   comprises ethanol.

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9. The invention of claim 1 or 2 wherein said cells
   are at risk of dying due to a neuropathy.

10. The invention of claim 9 wherein the etiology of
    said neuropathy is metabolic, infectious, toxic,
    autoimmune, nutritional, or ischemic.

11. The invention of claim 10 wherein said neuropathy
    comprises Parkinson's disease, Huntington's chorea,
    amyotrophic lateral sclerosis, multiple sclerosis
    or Alzheimer's disease.

12. The invention of claim 1 or 2 wherein said cells
    are at risk of dying due a neoplastic lesion
    associated with nerve tissue comprising said cells.

13. The invention of claim 12 wherein said lesion
    results from a neoplasm comprising cells of
    neuronal origin.

14. The invention of claim 13 wherein said neoplasm
    comprises a neuroblastoma or a retinoblastoma.

15. The invention of claim 12 wherein said lesion
    results from a neoplasm comprising glial cells.

16. The invention of claim 1 or 2 wherein said neural
    cells at risk of dying comprise part of the central
    nervous system.

17. The invention of claim 16 wherein said cells
    comprise striatal basal ganglia neurons.

18. The invention of claim 16 wherein said cells comprise neurons of the substantia nigra.

19. The invention of claim 1 or 2 wherein said cells at risk of dying comprise part of the peripheral nervous system.

20. The invention of claim 1 or 2 wherein said morphogen stimulates cell adhesion molecule production in said cells.

21. The invention of claim 20 wherein said cell adhesion molecule is a nerve cell adhesion molecule.

22. The invention of claim 21 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.

23. The invention of claim 1 or 2 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

24. The invention of claim 23 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).

25. The invention of claim 24 wherein said morphogen
comprises an amino acid sequence having greater
than 60% amino acid identity with the sequence
defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

5

26. The invention of claim 25 wherein said morphogen
comprises an amino acid sequence having greater
than 65% amino acid identity with the sequence
defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

10

27. The invention of claim 22 wherein said morphogen
comprises an amino acid sequence defined by
residues 43-139 of Seq. ID No. 5 (hOP1), including
allelic and species variants thereof.

15

28. A method for enhancing the survival of neural cells
at risk of dying in a mammal, the method comprising
the step of administering to said mammal an
effective amount of an agent capable of stimulating

20   production of an endogenous morphogen.

29. The method of claim 28 wherein said agent
stimulates production of an endogenous morphogen in
the tissue comprising said neural cells.

25

30. A method for maintaining a neural pathway in a
mammal, comprising:
    providing a morphogen to the neurons defining
said pathway at a concentration and for a time
30   sufficient to maintain said pathway.

31. The method of claim 30 wherein said morphogen is
provided prior to injury to said pathway.

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32. The method of claim 30 wherein said morphogen is sufficient to stimulate repair of a damaged neural pathway.

5   33. The method of claim 32 wherein said damaged neural pathway results from mechanical or chemical trauma to said pathway.

34. The method of claim 33 wherein said trauma
10   comprises a severed nerve.

35. The method of claim 33 wherein said trauma comprises demyelination of the neurons defining said pathway.

15
36. The method of claim 33 wherein said trauma results from exposure of the cells defining said pathway to a cellular toxin.

20 37. The method of claim 36 wherein said toxin comprises ethanol.

38. The method of claim 30 wherein said damaged neural pathway results from a neuropathy of the cells
25   defining said pathway.

39. The method of claim 38 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.

30
40. The method of claim 39 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, or Alzheimer's disease.

35

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41. The method of claim 38 wherein said neuropathy comprises axonal degeneration.

42. The method of claim 38 wherein said neuropathy comprises a demyelinating neuropathy.

43. The method of claim 30 wherein said damaged neural pathway results from a neoplastic lesion.

44. The method of claim 43 wherein said neoplastic lesion is caused by a neuroblastoma or a glioma.

45. The method of claim 30 wherein said morphogen stimulates cell adhesion molecule production in a cell defining said pathway.

46. The method of claim 45 wherein said cell adhesion molecule is a nerve cell adhesion molecule.

47. The method of claim 46 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.

48. The method of claim 30 or 45 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

49. The method of claim 48 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2,
5   Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).

50. The method of claim 49 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence
10   defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

51. The method of claim 50 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence
15   defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

52. The method of claim 51 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including
20   allelic and species variants thereof.

53. The invention of claims 1, 2, 30 or 46 wherein said morphogen comprises a polypeptide chain encoded by a nucleic acid that hybridizes under stringent
25   conditions with the DNA sequence defined by nucleotides 1036-1341 of Seq. Id No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.

54. The invention of claims 1, 2, 26, 30, 45 or 51
30   wherein said morphogen comprises a dimeric protein species complexed with a peptide comprising a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof.

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55. The invention of claim 54 wherein said dimeric
    morphogen species is noncovalently complexed with
    said peptide.

 5 56. The invention of claims 54 or 55 wherein said
        dimeric morphogen species is complexed with two
        said peptides.

    57. The invention of claims 54 or 55 wherein said
10   peptide comprises at least the first 18 amino acids
        of a sequence defining said pro region.

    58. The invention of claim 57 wherein said peptide
        comprises the full length form of said pro region.

15

    59. The invention of claims 54 or 55 wherein said
        peptide comprises a nucleic acid that hybridizes
        under stringent conditions with a DNA defined by
        nucleotides 136-192 of Seq. ID No. 16, or
20   nucleotides 157-211 of Seq. ID No. 20.

    60. The invention of claims 54 or 55 wherein said
        complex is further stabilized by exposure to a
        basic amino acid, a detergent or a carrier protein.

25

    61. A method of maintaining a neural pathway in a
        mammal comprising:
            administering said mammal an effective amount
        of an agent capable of stimulating production of an
30   endogenons morphogen in a cell defining said
        pathway.

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62. A composition for promoting regeneration of a
    neural pathway at a site of injury in a mammal,
    comprising:
        a biocompatible, _in vivo_ bioresorbable carrier
5   suitable for maintaining a protein at a site _in_
    vivo, and
        a morphogen, such that said morphogen, when
    dispersed in said carrier and provided to said site
    of injury, is capable of stimulating neural pathway
10 regeneration at said site.

63. The composition of claim 62 wherein said carrier is
    structurally sufficient to assist direction of
    axonal growth.

15

64. The composition of claim 63 wherein said carrier
    comprises a polymeric material.

65. The composition of claim 63 wherein said carrier
20 comprises laminin or collagen.

66. A device for repairing a break in a neural pathway,
    the device comprising:
        a biocompatible tubular casing comprising an
25 exterior and an interior surface and defining a
    channel through which a neural process may
    regenerate,
        said device having a shape and dimension
    sufficient to span a break in a neural pathway, and
30 having openings adapted to receive the ends of a
    severed nerve, and

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a morphogen disposed within the channel
defined by said tubular casing and accessible to
severed nerve ends defining a break in a neural
pathway, such that said morphogen stimulates neural
5   pathway regeneration when disposed in said channel
and accessible to said nerve ends.

67. The device of claim 66 wherein said morphogen is
disposed in said channel together with a
10   biocompatible, bioresorbable carrier suitable for
maintaining a protein at a site in vivo.

68. The device of claim 67 wherein said carrier
comprises sufficient structure to assist direction
15   of axonal growth within said channel.

69. The device of claim 67 wherein the outer surface of
said casing is substantially impermeable.

20 70. The device of claim 66 wherein said carrier
comprises a polymer.

71. The device of claim 67 wherein said carrier
comprises laminin or collagen.

25

72. A method for inducing the redifferentiation of
transformed cells of neural origin, the method
comprising the step of:
contacting said transformed cells with a
30   morphogen composition at a concentration and for a
time sufficient to induce redifferentiation of said
cells to a morphology characteristic of
untransformed neuronal cells.

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73. The method of claim 72 wherein said morphology
    characteristic of untransformed nerve cells
    includes formation of neurite outgrowths.

5 74. The method of claim 72 wherein said morphology
    characteristic of untransformed nerve cells
    includes cell aggregation and cell adhesion.

75. The method of claim 72 wherein said morphogen
10   composition induces nerve cell adhesion molecule
    production in said cells.

76. The method of claim 72 wherein said induced nerve
    cell adhesion molecules include N-CAM-180, N-CAM-
15   140 and N-CAM-120.

77. The method of claim 72 wherein said transformed
    cells comprise neuroblastoma cells.

20 78. A kit for detecting a neuropathy in a mammal or for
    evaluating the efficacy of a therapy for treating a
    neuropathy in a mammal, the kit comprising:
    c)   means for capturing a cell or body fluid
    sample obtained from a mammal;
25   b)   a binding protein that interacts specifically
    with a morphogen in said sample so as to form a
    binding protein-morphogen complex;
    c)   means for detecting said complex.

30 79. The kit of claim 78 which said binding protein has
    specificity for an epitope defined by part or all
    of the pro region of a morphogen.

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80. A method for detecting a neuropathy in a mammal,
the method comprising the step of:
detecting fluctuations in the physiological
concentration of a morphogen present in the serum
5   or cerebrospinal fluid of said mammal, said
fluctuations being indicative of an increase in
neuronal cell death.

81. A method for detecting a neuropathy in a mammal,
10   the method comprising the step of:
detecting fluctuations in the physiological
concentration of a morphogen antibody titer present
in the serum or cerebrospinal fluid of said mammal,
said fluctuations being indicative of an increase
15   in neuronal cell death.

82. The invention of claims 78, 80 or 81 wherein said
neuropathy results from a neurodegenerative
disease, nerve demyelineation, myelin dysfunction,
20   neuronal neoplasias, or nerve trauma.

83. A method of stimulating production of cell adhesion
molecules in a tissue comprising the step of:
providing a morphogen to said tissue for a
25   time and at a concentration sufficient to induce
production of cell adhesion molecules in cells of
said tissue.

84. The method of claim 83 wherein said cell adhesion
30   molecules comprises nerve cell adhesion molecules.

85. The method of claim 84 wherein said cells comprise
neurons.

86. The method of claim 78, 80 or 81 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

5

87. The method of claim 86 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A (fx).

10

88. The method of claim 87 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

15

89. The method of claim 88 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

20

90. The method of claim 89 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.

25

91. The method of claim 78, 80 or 81 wherein said morphogen comprises an amino acid sequence encoded by a nucleic acid that hydridizes under stringent conditions with the sequence defined by nucleotides 1036-1341 of Seq. ID No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.

30

35

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92. A composition for enhancing survival of neuronal
    cells at risk of dying comprising a morphogen in
    association with a molecule capable of enhancing
    the transport of said morphogen across the
5   blood-brain barrier.

93. The invention of claims 62 or 67 wherein said
    carrier comprises brain tissue derived
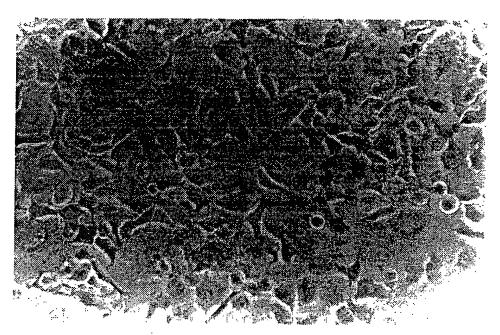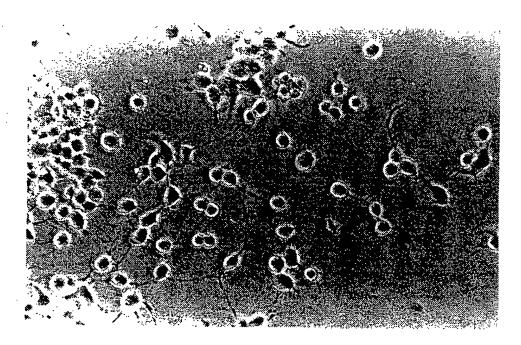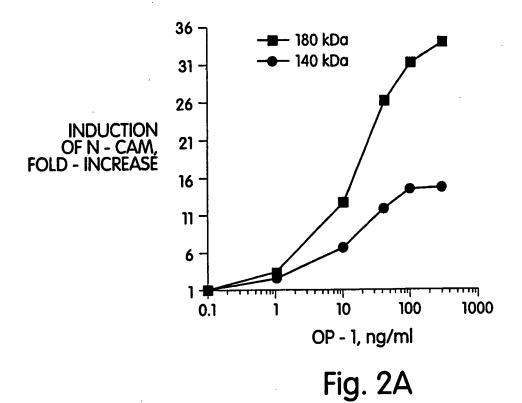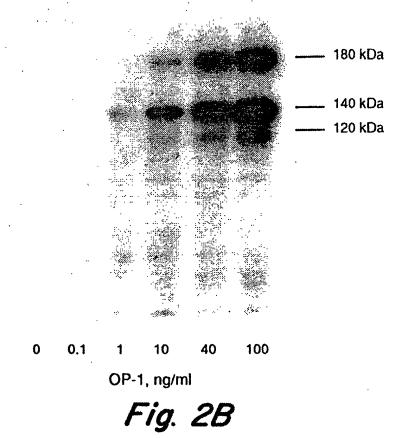   extracellular matrix.

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Fig. 1A

Fig. 1B

Fig. 2A

Fig. 3

Fig. 2B

Fig. 4

IPC 5   A61K37/02   G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5   A61K   C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 00382 (CARNEGIE INSTITUTION OF WASHINGTON) 9 January 1992 see page 9, line 15 - page 15, line 29 ---	1-24,78, 79,82, 86,87
X,P	WO,A,92 15323 (CREATIVE BIOMOLECULES, INC.) 17 September 1992 cited in the application see page 6, line 1 - page 26, line 18 ---	1-93
X,P	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89 , November 1992 , WASHINGTON US pages 10326 - 10330 GEORGE PERIDES ET AL. 'INDUCTION OF THE NEURAL CELL ADHESION MOLECULE AND NEURONAL AGGREGATION BY OSTEOGENIC PROTEIN 1' THE WHOLE ARTICLE ---	1,20-27, 53

-/--
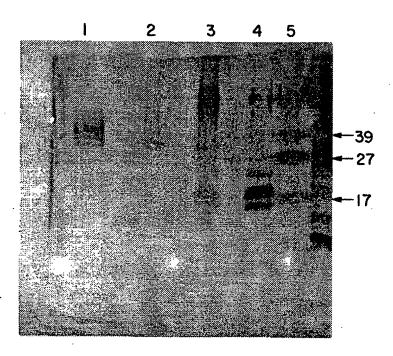
X	Further documents are listed in the continuation of box C.		X	Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 1993	0 7. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	REMPP, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOLOGICAL ABSTRACTS vol. 91 1991, Philadelphia, PA, US; abstract no. 106862, JONES, C. ET AL. 'INVOLVEMENT OF BONE MORPHOGENETIC PROTEIN-4 (BMP-4) AND VGR-1 IN MORPHOGENESIS AND NEUROGENESIS IN THE MOUSE' see abstract & DEVELOPMENT (CAMB) vol. 111, no. 2 , 1991 pages 531 - 542 -----	

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claims 2,28-52,61,72-77,80,81,83,85 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9200382	09-01-92	AU-A- 8496491	23-01-92
WO-A-9215323	17-09-92	AU-A- 1754392	06-10-92